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EFFECTS OF THE ABSENCE OF BORON AND OF SOME OTHER ESSENTIAL ELEMENTS ON THE CELL AND TISSUE STRUCTURE OF THE ROOT TIPS OF *PISUM SATIVUM*

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(WITH FIVE PLATES)

Introduction

While the rôle of the various elements in the growth of the plant has attracted the attention of a large group of workers since the beginning of modern botany, the real function of certain of these elements still remains one of the least understood phases of plant physiology.

Relatively early in the history of the investigation of the growth of plants in culture solution, certain elements were found to be "essential" while others appeared to be non-essential for the normal development of the organism. Obviously, however, it is far easier to demonstrate that an element which is required in relatively large quantities is essential than to prove that elements are not necessary at all. In consequence of the difficulty of establishing the need of plants for certain elements which are required in very small quantities, the list of the "essential" elements was very short, while by implication the list of "non-essential" elements included all the rest.

At the present time the more refined purification of the so-called "chemically pure" salts, and more carefully controlled methods of physiological research, permit a study of the effects of the absence from culture solutions of certain elements with greater exactness than before, with the result that elements once considered unnecessary are being shown to be indispensable for the normal development and functioning of the plant.

The present study forms a part of the work on the essential nature of boron and certain other elements for higher green plants which one of the

writers (**7, 8, 9**) has been carrying on for the past three years. It represents, however, a cooperative extension of this work into a relatively new field.

The great bulk of the research on the influence of the different essential elements on the development of the plant has employed, as a sole criterion, the mass of tissue produced by plants grown in solutions of varying composition and concentration. This represents only a limited front of attack on the general problem. An ultimate goal should be the determination of the actual rôle of each of the elements in the metabolism of the plant, and the consequent influence of these elements on the morphology of the cell and of the tissues.

While a study of the structure of the cell and the tissues can not furnish final criteria of the rôle of the various elements in the metabolism of the plant, it may represent the first practicable steps toward this end. The work upon which this paper was based was undertaken with this end in view.

Boron seems an unusually favorable element to be employed in a first study of this kind. It was early noted (**8**) that many dicotyledonous plants when grown in solutions without boron developed short thick roots with enlarged apices. It seemed, therefore, that a study of the pathological changes which take place in these cells and tissues might give some clue as to the function of boron in plant growth.

Both the chemical and cytological phases of such a problem demand refined, delicate and specialized technique. The interpretation of the results requires a knowledge of two fields of literature. At the suggestion of Dr. J. Arthur Harris, whose interest in and encouragement of this work we have greatly appreciated, we therefore undertook a cooperative investigation of the problem.

Pisum sativum (variety Golden Vine) was chosen as the experimental plant because it was found to show the effects of the absence of boron within a few days and also to produce root tips of a very suitable size for cytological work.

In first experiments plants were grown in a satisfactory culture solution to which boron was or was not added. This solution was the same as that described later as the control solution.

As work on the problem progressed it became evident that a study of the effect of the absence from the culture solutions of some other of the essential elements would be extremely desirable, since such experiments might throw some light on the problem involved. Therefore in a series of cultures most of the elements essential for plant growth were excluded one at a time from the solutions, and the effects of the absence of the various elements in the absence and presence of boron were studied.

Methods

Culture methods

All plants were grown in solutions of highly purified salts. Quart Mason jars were usually employed as containers. In a few instances pyrex breakers were used. The exceptional cases will be discussed later.

This investigation included cultures with and without each of the following elements: boron, magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium. Each of the last eight groups included cultures with and without boron.

Although zinc was shown by Mazé (**3**) to be essential for maize and by one of us (**7, 9**) to be essential for several other plants, it was not included in this investigation which deals primarily with the histology and cytology of the root, because of the fact that, in the absence of zinc, the roots appear normal even after the tops are apparently dead. The results caused by the absence of calcium were so striking and so different from anything published so far that they are being reserved for another paper which is ready for publication.

The cultures without potassium were divided into two subgroups, one in which potassium was replaced by sodium and one in which no sodium was added. Pyrex beakers were used as containers in experiments omitting sodium to preclude the possibility of the introduction of this element by solution from soft glass. The purpose of this division was to determine whether any effect of the replacement of potassium by sodium could be observed. In other experiments without potassium (where the effect of sodium was not being studied), Mason jars were employed.

Solutions used were:

Control solution

	Per liter		Gm. per liter
KNO_3	0.80 gm.	Mn (as $MnSO_4$)	0.0015
KH_2PO_4	0.15 gm.	Al (as $Al_2(So_4)_3$)	0.0005
$MgSO_4 \cdot 7H_2O$	0.50 gm.	Cu (as $CuSo_4$)	0.000125
$CaSO_4$ saturated solution	300 cc.	I (as KI)	0.00025
B (as H_3BO_3)	0.0005 gm.	F (as KF)	0.00025
		NaCl	0.0127

Iron (as $FeSO_4$) was added at intervals as required by the plants. Manganese, iron, and boron were omitted from the solution given above in studies concerning the absence of these elements.

Solution without magnesium

	Per liter	
KNO_3	1.20 gm.	B, Mn, Al, Cu, I, F and NaCl as in control solution.
KH_2PO_4	0.15 gm.	
$CaSO_4$ saturated solution	300 cc.	

SOLUTION WITHOUT SULPHUR

	Per liter	
KNO_3	1.00 gm.	Traces of the various elements were added as chlorides or nitrates instead of sulphates. Fe as citrate.
$Ca(NO_3)_2$	0.60 gm.	
$MgHPO_4$	0.20 gm.	

SOLUTIONS WITHOUT POTASSIUM

I

	Per liter	
$NaNO_3$	1.00 gm.	Traces of various elements as in control excepting that I was added as NaI.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

II

	Per liter	
$Ca(NO_3)_2 \cdot 4H_2O$	1.2 gm.	Traces as above.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

SOLUTION WITHOUT NITROGEN

	Per liter	
KH_2PO_4	0.15 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	
K_2SO_4	0.80 gm.	

SOLUTION WITHOUT PHOSPHORUS

	Per liter	
KNO_3	0.80 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	

PURIFICATION OF SALTS

KNO_3, $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$, Na_2SO_4, $MnSO_4$ and H_3BO_3 were repurified by repeated recrystallization from pure distilled water. $CaSO_4$ was prepared by dissolving chemically pure $Ca(NO_3)_2$, filtering and precipitating the $CaSO_4$ with chemically pure H_2SO_4. The $CaSO_4$ was then washed free from acid. $FeSO_4$ was prepared by dissolving iron wire (as used for standardization) in chemically pure H_2SO_4, filtering and crystallizing. $MgHPO_4$ was prepared from purified K_2HPO_4 by precipitating with purified $MgSO_4$. The precipitate was then washed many times with pure distilled water. With the exception of $MnSO_4$ and H_3BO_3 none of the substances used only in very small amounts was repurified. NaCl was used in relatively larger quantities but P. W. R. (Powers-Weightman-

Rosengarten) analyzed salts appeared to be pure enough, so this salt was not purified further. $NaNO_3$ was prepared by evaporating pure NaCl with redistilled HNO_3 until all the chlorine was driven off. The $NaNO_3$ was then crystallized. In the investigations without sulphur it was very difficult to exclude boron when this was desired. $FeSO_4$ seemed to be the only soluble salt easily freed from boron, and the lack of boron was shown in sulphur-free solutions only when a minimum amount of iron as the citrate was added.

No test of the purity of the salts was made other than that shown by the ability or failure of plants to develop in solutions made with them but without the addition of boron. This procedure was followed because we have found that many plants respond to smaller quantities of boron than can be determined chemically and because, according to AUER-WELSBACH (1), small amounts of this element are very difficult to detect spectroscopically in the presence of relatively large amounts of other elements.

HISTOLOGICAL AND CYTOLOGICAL METHODS

In obtaining the material for the present investigation, two important precautions were observed. First, environmental factors which may influence the structure and general appearance of the cell components were made as nearly alike as possible and, second, an effort was made to take the tissues for fixation under the most normal conditions during periods of most active nuclear division of the meristematic cells. The material was, therefore, always taken on bright days, in the morning between eight and twelve o'clock. In all cases, with the exception of those mentioned separately, the roots were killed after the plants had been in corresponding culture solutions for from two to three weeks. The majority of the experimental series were repeated during different times of the year.

As a killing reagent, NAWASCHIN's modification of FLEMMING's killing solution was used throughout the investigation. As previous experience of one of the writers indicates, this fixing agent is very reliable and gives splendid results with tissues and cell components. Subsequent treatment was that usually required by and given to cytological preparations. Longitudinal sections of the roots were cut 12 μ and 15 μ thick, and were stained with safranin and aniline blue or with HEIDENHAIN or DELAFIELD haematoxylin.

The photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken with exactly the same magnification (about 45 diameters).

Results of histological and cytological studies

Plants grown in culture solutions with and without boron are shown in fig. 5, plate VIII. The three plants to the left were grown with boron for about three weeks; those to the right were grown without boron for the same length of time.

Plants grown in standard culture solutions with and without boron

With boron, fig. 1, plate VII.—The apical portion of roots of *P. sativum* grown in culture solutions in the presence of boron show the typical differentiation of the primary meristem into three distinct regions, plerome, periblem and dermatogen. In the region of contact with the promeristem, the root cap is very well developed and is usually about 20 cells in width. The plerome is about 12 to 14 cells in diameter, the cells being 11 μ x 18 μ, and the nuclei about 10 μ x 10 μ. The nuclei occupy a central position in the cell and each contains a conspicuous nucleolus. Mitotic figures are very numerous in both the plerome and the pleriblem. The dermatogen is usually not very much differentiated from the periblem. The diameter of the root tip in the middle of the region of the primary meristem is approximately 28 to 32 cells. Starting with the beginning of the promeristem, the region of intensive cell division extends for a distance of about 20 to 25 cells to the beginning of the region of elongation. The region of maturation usually does not begin at a distance of less than from 60 to 70 cells from the tip.

Without boron, figs. 2–4, plate VII.—The apical portions of roots grown in culture solutions containing the elements usually considered essential, but without boron, show remarkable changes from those described above. The region which corresponds to the primary meristem of the normal root can hardly be called meristem. Progressive changes (in the sense in which this term is used by students of pathological plant anatomy) take place, which entirely modify the appearance as well as the structure of this region. Since the term meristem cannot be properly applied to the region under discussion, it would be logical to avoid the terms plerome and periblem in indicating that portion of the root in which pathological changes take place. But because the terminology of pathological plant anatomy lacks uniformity in general and is entirely inadequate for this special case, we will refer to the regions found in the apical part of roots grown without boron as plerome, periblem, and dermatogen, always keeping in mind the fact that these names are used here, not for regions of primary meristem, but for those homologous with the primary meristem in the normal root.

In the absence of boron the root cap is considerably deformed or even entirely lacking. In the region of the plerome and periblem progressive

changes take place which result in an enormous enlargement of the whole apical portion of the root. Distinct hyperplasia is developed in the plerome, while in the periblem hypertrophy usually obtains. In the central portion of the apical part of the root the plerome region usually extends from about 30 to about 32 cells in diameter, which is about twice as many cells as are present in the corresponding region of roots grown with boron. In the early history of the cultures the cells divide by mitosis but soon lose their ability to divide further by typical mitosis. In the absence of boron the regularity in the arrangement of the cells is lost. The mechanism regulating the divisions of the cells is apparently disturbed, with the result that the cells appear in irregular uncoordinated rows or even without the formation of definite rows. The cells are extremely irregular in shape and size. They range from 10 μ x 15 μ to 11 μ x 18 μ, and the nuclei are about 6 μ x 7 μ.

The rows of cells in the periblem regions are more regular than in the plerome and correspond in number to those of the normal root. The cells, however, are much larger than normal, being 50 μ x 50 μ with nuclei 7.5 μ x 8 μ. It is interesting to note that although these cells are much larger than the cells of the normal root, their nuclei are smaller. Returning to the plerome we note that besides the lack of regularity of arrangement and form of the cells, another interesting abnormality appears. The cells of this region undergo premature development which is manifest in the appearance of isolated elements of xylem as far down as the level of the twentieth cell from the apex. These first occur at irregular intervals and show unequally thickened walls which do not take the stain typical for lignified cell walls. The elements occurring slightly farther up stain red with safranin but are extremely irregular in appearance. Incompletely scalariform or scalariform-reticulate xylem elements usually predominate. Besides the abnormal occurrence of these xylem units, the premature development of the tissues is further manifest in the formation of isolated meristematic regions of branch root primordia in different parts of the apical portion of the root. These regions are sometimes formed at a distance of no more than 10 to 13 cells from the apex. However, they very rarely continue their development for any length of time, but are apparently suppressed shortly after they have been formed. This is illustrated in fig. 4, plate VII. The photograph presents a tangential section in which a series of new meristematic regions is shown, most of which were suppressed soon after they were formed. Only the fifth of these incipient lateral roots developed far enough to grow through the periblem. A careful examination of the material shows that typical mitoses are usually absent. In these newly formed meristematic regions, slightly abnormal mitoses are observed.

Another important characteristic of the tissue found in the plerome region is the formation of thick strands which apparently represent the collapsed and thickened walls of cells. These are shown clearly in fig. 3, plate VII. Farther away from the apical portion, a region occurs in which the lignified elements become more prominent and are even connected for more or less regular distances. These, however, may be interrupted by portions in which the connection between the individual elements is lacking. Pitted vessels begin to be more prominent in this region.

Recovery from injury due to the lack of boron

Root tips in the process of recovery from injury due to the lack of boron present a very interesting picture. If boron be added to solutions which have produced plants showing typical symptoms of the lack of boron, but whose tissues are not too badly disintegrated, evidences of recovery appear very quickly.

In a series of experiments boron was added to solutions in which the plants showed an extreme degree of injury due to the lack of this element, and root tips of these cultures were fixed after 6, 24, and 48 hours. A definite zone of truly meristematic cells seems to appear as early as 6 hours after the addition of boron. A newly formed meristematic region is apparent in the tissues fixed at the end of 24 hours. After 48 hours a new long slender growth may be seen at the end of the enlarged apex. This newly formed root apex sometimes grows more than a centimeter in 48 hours, and acquires the diameter and general appearance of the normal root.

Plants grown in solutions without magnesium

With boron, fig. 6, plate VIII.—The root apices of plants grown in solutions lacking only magnesium do not show any noticeable deviation from the normal. Mitotic figures are numerous and regular.

Without boron, fig. 7, plate VIII.—Rather distinct peculiarities were observed when both boron and magnesium were absent which were not seen when boron alone was lacking. We cannot say that these were real differences, and not variations of the effects of the absence of boron alone, since sections of only three root tips were available for study. It is, however, desirable to describe these differences which are as follows: the plerome and periblem regions are less definitely marked off in the most apical portion of the root than those of cultures grown without boron alone. Hypertrophy sometimes occurs in the plerome as well as in the periblem. There are also some other deviations from the typical conditions for the absence of boron. The isolated regions of branch root primordia are not so prominent. The cells of the periblem region appear less hypertrophied than

those of the corresponding region of roots of plants grown without boron only, their size being 25 μ x 25 μ instead of 50 μ x 50 μ. The size of the nuclei is, however, the same in both cases.

Plants grown in solutions without sulphur

With boron, fig. 8, plate VIII.—The roots appear more or less normally developed with the exception of the occurrence of rather large intercellular spaces in the periblem region, where whole rows may be missing. A similar phenomenon sometimes occurs normally in certain species of plants but we have not observed it in roots of *P. sativum*. Sometimes single cells are lacking, making the rows discontinuous. This apparently does not occur in the plerome region where the cells appear very normal, and where mitotic figures occur. The size of the cells in the plerome corresponds to those for plants grown in the control solutions.

Without boron.—In our first experiments, where an attempt was made to exclude both boron and sulphur we failed to produce the typical effects due to the lack of boron. Contamination of the solution by boron was suspected, since $FeSO_4$ is the only soluble iron salt easily purified and this could not be used because it would add sulphur which we also wished to exclude.

In earlier experiments in which there was probably some contamination by boron, the roots did not differ much from those grown in solutions to which boron had been added, but the intercellular spaces observed in the periblem region when only sulphur was lacking were even more conspicuous. This is well shown in a tangential section of a root, fig. 9, plate VIII. In a later experiment, in which only a very limited supply of iron (as ferric tartrate) was used, and the contamination by boron thus greatly reduced, an approach to the typical effects due to the absence of boron is easily observed.

Plants grown in solutions without manganese

With boron, fig. 11, plate IX.—The size of the root and the regions of meristem is normal. Mitotic figures are numerous and normal. In the region of elongation the cell walls are, however, abnormal, being irregularly thickened in certain parts and loosely coherent in others. A considerable thickening of the transverse cell wall is particularly noticeable.

Without boron, fig. 10, plate IX.—In contrast with other cultures grown without boron, roots grown without this element and without manganese have well developed root caps in all cases which were studied. In the meristematic region of the primary root, the nuclei divide by mitosis, but some irregularities in the process of division are observed. The metaphases appear very much deformed. An orientation of the spindle nor-

mally parallel to the longitudinal axis is seldom observed. Figures in which anaphases and telophases can be recognized are almost entirely lacking in the root apices, but reduced anaphases and telophases are present in the secondary roots. These stages seem, however, to occur only during the early period of the development of the laterals. There is some evidence that, after the plants have been growing for a considerable length of time in the absence of manganese and boron, the nuclei of the cells of the principal root apex begin to divide by pseudoamitosis, or fail to divide at all. This may, however, be a general phenomenon common to the absence of several of the essential elements.

The pathological changes of the tissues consisted in considerably developed hyperplasia of the cells of the plerome, and hypertrophy of the cells of the periblem. The premature development of the cells is very pronounced, the isolated and incompletely developed xylem elements appearing early. The formation of secondary roots occurs very close to the meristematic region. The cell walls appear to be extremely thick in certain cells, which is clearly shown in the periblem of the upper part of the photomicrograph, fig. 10, plate IX.

Plants grown in solutions without potassium

As mentioned in the paragraph describing the solutions used in this investigation, groups of the plants in the work with potassium were employed in order to determine whether there was any visible effect of the replacement of potassium by sodium. The cytological and histological pictures were the same in both cases, so what is said for one solution holds for the other also. Pyrex containers were used for these cultures to prevent the introduction of sodium from the glass. Since pyrex is a borosilicate, enough boron was supplied to the boron free solutions from the containers to prevent the symptoms of the lack of boron from appearing for some time. Within a month, however, plants growing in solutions in pyrex containers to which no boron had been added began to show the characteristics produced by the absence of boron. In experiments in which ordinary Mason jars were used, the effects of the absence of boron appeared in the early stages of growth.

With boron, fig. 12, plate IX.—The roots exhibited normal differentiation of the meristematic tissue into plerome, periblem, and dermatogen. The root cap was very well developed and typical mitoses occurred frequently in the meristem. It differed from the control in that there was a somewhat loose arrangement of the cells in certain parts of the roots and the occasional dropping out of entire rows. These results differ from those obtained by Reed (**5**) for *Spirogyra* which was cultured in the absence of potassium and in which mitotic divisions were not observed. His plants

were kept in potassium free solutions for thirty-five days, while ours were without potassium other than what was stored in the seed for less than three weeks. Whether mitotic division in his *Spirogyra* plants ceased because other processes had been too adversely affected, whereas ours had not yet reached this stage, or whether these very different types of plants respond differently to the absence of potassium is a matter for further investigation.

The rôle of potassium in the metabolism of the cell has been studied by MACALLUM (**2**), and WEEVERS (**11**). See MOLISCH (**4**), pp. 63–64. These authors succeeded in demonstrating, by microtechnique, the presence of this element in the cytoplasm of cells of all plants investigated except in those of the *Cyanophyceae*. Their reaction for potassium in the nucleus was negative, and from this fact they concluded that potassium as a rule is absent from the nuclei. Their test, however, would not show potassium in organic combination, and although we do not know that such compounds exist in the plant, we still have to consider this possibility.

Laws of physical chemical equilibrium make it appear impossible that the potassium ion would be entirely absent from the nucleus when it is present in the surrounding cytoplasm, and furthermore, a colloidal substance with such elasticity as SEIFRIZ (**6**) has shown the nucleus of *Cryptobranchus* to have, would very probably prevent the precipitation of the potassium compound in particles large enough to be seen with an ordinary microscope.

In addition to the results in the papers mentioned above, WEEVERS (**11**) found that potassium was especially abundant in the cell vacuole and absent in the chromatophores. Potassium was conspicuous in the growing points and storage organs of the phanerogams. The reaction showed plainly in the sieve tubes, but poorly in the other vascular elements. A considerable amount of potassium was demonstrated in the cambium and medullary rays. MOLISCH (**4**), p. 64, comes to the general conclusion that, although it is difficult as yet to decide what the exact rôle of potassium in cell metabolism may be, there are many indications that this element plays a part in the building up of the plasma of the growing points, and that it is important in the development of turgor in the cells.

WITHOUT BORON, FIGS. 13, PLATE IX, and 14 and 15, PLATE X.—The typical pathological changes due to the lack of boron in the series of experiments from which both boron and potassium were excluded were not obtained at once in the first experiment. The difficulty may be attributed to the fact that in attempting to grow plants in pyrex containers sufficient boron may be introduced from the glass for normal development during the early stages of growth. Another difficulty was that the salts used in this experiment were not quite free from boron. Finally both difficulties were over-

come and a typical demonstration of the abnormalities due to the absence of boron was obtained.

A longitudinal section of the apical portion of a root is represented in fig. 13, plate IX. The meristematic region is abnormal but not entirely abortive. It is represented in the photomicrograph as a very dark area. The nuclei show division by mitosis, in which all of the phases were observed, but there is a deviation from normal mitosis in the somewhat irregular appearance of the chromosomes. Hypertrophy and hyperplasia of the periblem and plerome respectively were distinct, but not as extensive as in the cultures lacking boron only. The region showing hyperplasia was only 22 cells in diameter as compared with 30 to 32 cells in the corresponding region of root tips grown in the absence of boron only. The premature development of the root regions was not as distinct as in the cultures lacking boron alone. The lignified elements of the xylem, as represented in fig. 13, are at a region less than two thirds of the distance from the apex as compared with one sixth of the distance where boron alone was excluded. The abnormal formation of branch root primordia was not observed. Root apices from plants grown in solutions from which both boron and potassium were excluded appeared less deformed than those of plants in which only boron was absent. Here a slight contamination of the culture medium may explain the differences.

A longitudinal section of the apical portion of a root grown in a pyrex container for two weeks is shown in fig. 14, plate X. There is practically no difference between this root and those grown in the control solutions. A root from the same culture which was grown for a month and a half is represented in fig. 15, plate X. Although it is not a typical picture of a root grown in the complete absence of boron there is considerable resemblance. Pyrex glass evidently yielded sufficient boron for normal development in the early stages of growth but not enough to supply the plants for continued development.

Plants grown in solutions without nitrogen

With boron, figs. 16 and 17, plate X.—The typical characteristics of roots of pea plants grown in solutions without nitrogen but in the presence of all other essential elements is that they are comparatively long and of small diameter. Sections of the same root are shown in figs. 16 and 17. The section represented by fig. 16 is not in immediate continuation of that given as fig. 17, there having been omitted from between these two sections another part as long as that in fig. 17 and similar in structure to the upper part in fig. 17.

The root cap is very poorly developed. The meristematic region is present and the mitotic figures are distinct, but the cells appear disarranged,

especially in the periblem region, where large intercellular spaces may be observed. The general orientation of the rows is apparently very much affected by the absence of nitrogen. The region of mutation appears to be present at a normal distance from the root apex. The elements of the xylem appear to be connected in the regular manner. The secondary roots, when formed, are deflected downward to an unusual degree and destroy the cortex not only where they protrude but also in the region immediately surrounding this part. The cells of the plerome region are 10 μ x 20 μ; the nuclei are 10 μ x 10 μ. The nuclei are regular and spherical and appear normal. In the periblem region, the cells are about 30 μ x 15 μ, very irregular and in sections often without visible connection with the neighboring cells. The nuclei, however, are not diminished in size and are similar to the nuclei of the plerome region.

WITHOUT BORON, FIGS. 18 and 19, PLATE X.—The differentiation of the regions which are more or less distinct in case of plants grown without nitrogen and with boron, is less obvious in the roots of plants grown without boron. As a rule the progressive tissue changes which occur in these roots generally take the form of hyperplasia, which in this case occurs in the region corresponding to the plerome as well as the periblem. The cells are very small and their number correspondingly great. In certain parts of the root apices the diameter is more than 50 cells in width as compared with 28 to 32 cells in the normal root and 46 to 50 cells where boron only is absent.

The cells which undergo hyperplasia are very small and extremely irregular in size, ranging from 5 μ x 7 μ to 10 μ x 10 μ. Many of the cells could not be measured because their outlines were so indefinite. The nuclei are very small in the region of hyperplasia, being about 4 μ x 5 μ. In the parts where a slight differentiation into regions of primary meristem have taken place, the cells of the periblem are 15 μ x 22 μ and the nuclei are 6.25 μ x 6.25 μ.

In contrast to the roots of plants grown without nitrogen but with boron, the cells of the periblem region, when such a region becomes differentiated, occur in more or less regular rows. The tissue of the plerome, however, shows extreme irregularity in the arrangement of the cells. The typical rows could be distinguished very rarely and the cells varied greatly in size and shape. In a few cases, however, as represented in fig. 18, plate X, the hyperplasia was not developed to so great an extent, and hypertrophy was more pronounced.

PLANTS GROWN IN SOLUTIONS WITHOUT IRON

WITH BORON, FIG. 21, PLATE XI.—The root tip did not differ from the control; mitotic figures were numerous and normal. The size of the cells and of the nuclei was the same as that of the controls.

WITHOUT BORON, FIG. 20, PLATE XI.—Root tips of plants grown in solutions without iron and without boron showed the same characteristics as those of plants grown in solutions from which boron only was absent. No mitoses were observed in the apical part of the root, but well developed mitoses were present in the meristematic region of the secondary roots, as shown in fig. 20, plate XI. A few well developed metaphases and other stages of nuclear division are present even in the central cylinder of the primary roots.

PLANTS GROWN IN SOLUTIONS WITHOUT PHOSPHORUS

WITH BORON, FIG. 22, PLATE XI.—The external form of the roots of plants, grown for from two to three weeks in solutions without phosphorus but with boron, does not appear very much modified in comparison with normal roots. A study of the tissues showed that the arrangement of the cells was regular. Within the cells, however, the abnormalities were pronounced. The same fixing fluid which proved to be so excellent for all other cases studied apparently gave sections exhibiting plasmolysis. We have as yet no means of telling whether this condition was caused by the fixing agent or whether it occurred before the tissue was killed.

The nuclei are the structures most affected by the absence of phosphorus. They lose their typical more or less spherical shape and assume an extremely irregular outline. Amoeboid, elongated, spindle shaped and constricted nuclei are very often found in the meristematic region. Some of the nuclei are much smaller than those of normal cells. Instead of occupying a position in the center of the cell, as is characteristic of nuclei in meristematic tissue, they often appear flattened against the transverse cell wall. Normal mitotic figures were not observed. The nuclei sometimes appeared more normal in the region of elongation of the root.

Somewhat similar effects on the cells of lower plants have been described by REED (**5**). He found that not only were mitoses absent in plants of *Spirogyra* which were growing for three weeks in phosphorus free solutions, but that the cells grown for a certain length of time in such solutions were difficult to stimulate to divide when they were placed in solutions containing phosphorus. According to REED, cells transferred to phosphorus free solutions first lose the soluble phosphorus compounds and later show injury in the living parts of the cells.

In our experiments changes in the root tips of the peas described above took place in plants which were kept in phosphorus free solutions for from two to three weeks. Plants kept under similar conditions for a shorter time showed mitotic divisions of the nuclei which were normal or only slightly abnormal. The fact that peas can be grown in phosphorus free solutions for as long as four or more weeks is difficult to explain in

view of the fact that plants with comparatively minor injuries to the cells caused by the absence of boron die in so short a time.

It must be remembered, however, that the influence of the absence or presence of a given element can not be judged wholly by its morphological effects on the cell and tissue structure. The effect of boron on the cells and tissue structure may not be fully represented by abnormalities of structure. This influence may be merely secondary, and its real function concerned directly or indirectly with some very vital and more continuous process, such as, for example, respiration.

The whole problem of the effects of the absence of phosphorus, as well as of the absence of many of the other essential elements, requires much additional investigation.

WITHOUT BORON, FIG. 23, PLATE XI.—Pathological changes in the tissues of plants grown in the absence of both phosphorus and boron resemble those described for plants lacking boron only. The cells of the central cylinder seem to be most injured. Their protoplasts disintegrate. The process of disintegration ultimately results in the complete dissolution of the nucleus and cytoplasm. The disintegration of certain individual cells results in an irregularity of the arrangement of the cells of the central part of the root. The nuclei which retain a nucleolus, even though it may be very inconspicuous, remain alive. These nuclei, however, differ from those typical of meristematic tissues in that they resemble a coarse precipitate instead of the finely granular structure of the typical resting nucleus.

Discussion

In conducting the present investigation our primary object was to determine the changes in the cells and tissues of the root tips of *P. sativum* induced by the absence of boron from the culture solutions. To make these tests more critical, certain other elements recognized as essential, namely magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium, were omitted one at a time from the media. These studies, in which elements other than boron were excluded, were carried out only to determine whether or not their absence would influence the typical effects induced by the absence of boron. They are, therefore, very incomplete in so far as the specific effect of their absence is concerned. Each of these elements deserves an extensive, independent investigation from the cytological and histological view point, which, because of the purpose of this problem, was not included in this paper. We feel, however, that the results obtained here incidental to the study of the absence of boron, are of sufficient importance to warrant their being recorded as a basis for future special investigations of the structural changes occurring in the absence of these several elements.

Studies of the effects of the absence of boron in the presence and absence of certain of the other essential elements have shown the structural modifications to be roughly similar for all the elements with the exception of calcium. The absence of calcium caused such marked and rapid changes of the tissues, and its effect anticipated that of the absence of boron, so that if the absence of boron caused any abnormality it could not be observed when both elements were wanting. The remarkable effects of the absence of calcium will be treated separately in a subsequent paper.

Meristematic tissues are the first to show the effects of the absence of boron. On the exclusion of boron from the medium, the factors regulating the process of cell division and the normal orientation of the cells in the tissues no longer function normally. In a relatively short time after the plants are transferred to boron free solutions the cells cease dividing and a general arrest of growth follows. Thus the absence of boron influences not merely the structure of the cell but the organization of the tissue. Although more or less regular mitotic divisions are present in the early stages of root development of plants grown without boron, some modifications of this process occur very soon and result in pathological tissue changes. Hyperplasia becomes very pronounced in the region which corresponds to the plerome of the normal root while a distinct hypertrophy is observed in the periblem. The suppression of mitotic divisons in the absence of boron is not typical for the cells of the whole root. Secondary meristematic regions may be formed in the more apical part of the root. These are, however, usually suppressed during an early stage of development, and true secondary roots seldom develop. Normal mitoses occur at the beginning of these new meristems but soon disappear.

The changes in the anatomical structure of the roots of *Vicia faba* induced by the absence of boron were studied by Miss WARINGTON (**10**). She found that the cells of the cambium were affected first. These cells were shown to lose their regular outline, and peculiar dark streaks were found between them. Their development was also irregular, a few large cells often occurring in place of a number of small ones. This was particularly noticeable in the region of the cambium, and according to Miss WARINGTON's interpretation, was partly due to the breaking down of some individual cells rather than to any enlargement of existing cells. She also noticed the suppressed development of branch root initials, which, owing to the injury sustained from the lack of boron, failed to develop normally. She interpreted the thickened appearance of the roots as due to the irregular development of the pericycle and the formation, but suppressed development, of the root initials above mentioned.

We cannot fully agree with Miss WARINGTON's explanation of the cause of the thickened appearance of the root. The principal cause, ac-

cording to our investigation is the hypertrophy of the periblem and hyperplasia of the plerome regions. The formation of the suppressed root initials may, of course, add some additional thickness, which is usually seen as spherical enlarged areas in the stunted root apex.

The changes in root tips of *P. sativum* induced by the complete or, perhaps better, the nearly complete absence of boron from the culture solution, take place so rapidly that our study is chiefly one of final results. This makes interpretation difficult. It is obvious that a factor or factors regulating growth have been disturbed, but it is impossible, with only this final morphological picture to decide whether this is a direct or an indirect effect. Studies of root tips taken at relatively short intervals after the plants have been transferred to boron free solutions may help in solving the problem by providing a more or less complete series of the intermediate stages.

Another line of attack is suggested by the fact that certain plants, namely the grasses (**8**), (**9**) respond quite differently to the absence of boron from the nutrient media. We are inclined to believe that such differences in response cannot be due only to the amount of boron stored in the seed, but that they are probably concerned with some marked difference in the metabolism of the different plants. We hope that a more extensive comparative study of different forms will give a clew as to what part boron plays in the development of the organism.

Summary

1. Plants of *Pisum sativum* grown in solutions in the absence of boron exhibit short, thick, and stunted roots.
2. Plants grown in culture solutions in the absence of boron and of certain of the other essential elements showed pathological changes roughly similar to those found in the absence of boron only.
3. The enlargement of the root apices is due to the hyperplasia of the plerome and hypertrophy of the periblem regions.
4. The meristematic region of root tips grown without boron becomes abnormal. The cells cease dividing normally and existing cells undergo premature development or pathological changes. Isolated xylem elements appear in regions occupied by the meristem in normal roots, or in the region of elongation.
5. The primordia of the secondary roots begin to be formed abnormally close to the root tip. Usually, however, they are soon suppressed and secondary roots seldom develop.
6. In a general way, we may state that the absence of boron causes a disturbance in the regulation of growth and development.

7. The profound structural changes here observed make it quite clear that physiological investigations of the various elements must be accompanied by morphological, histological and cytological studies to attain their fullest significance.

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EXPLANATION OF PLATES

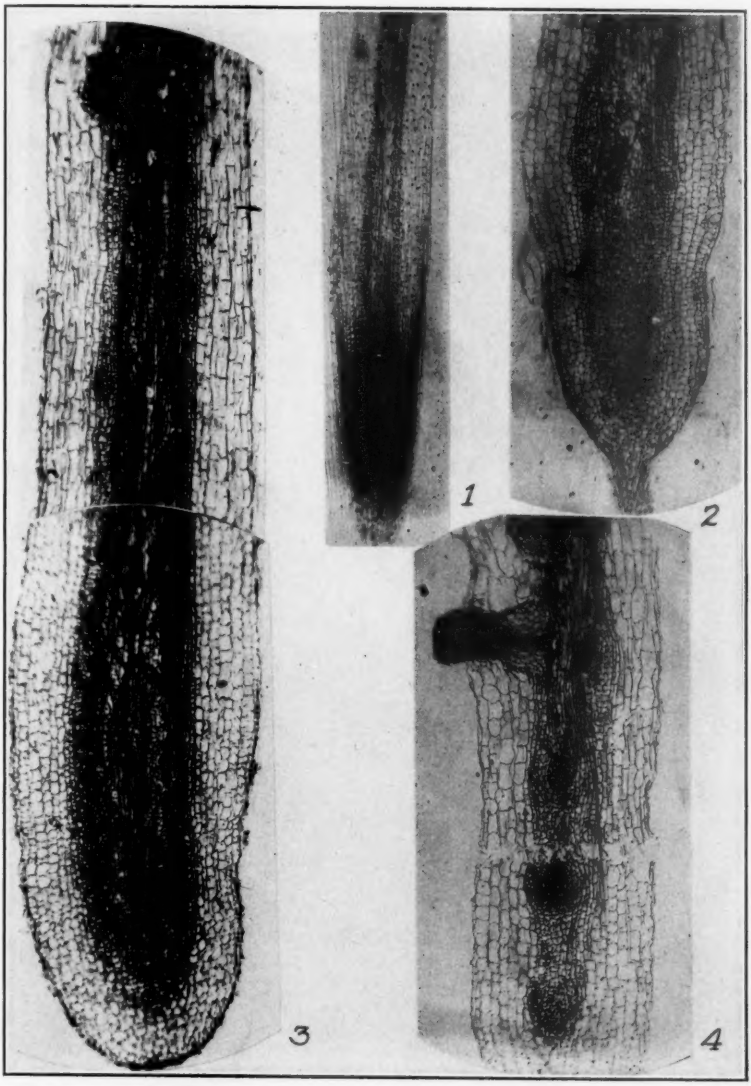
All photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken at the same magnification. The experimental plant was *Pisum sativum* (variety Golden Vine). All figures excepting 5, plate VIII, represent longitudinal sections of root apices.

FIG. 1. Grown in control solution; all elements present.

FIG. 2. Grown in the absence of boron.

FIG. 3. As in figure 2. Shows hyperplasia of plerome and hypertrophy of periblem.

FIG. 4. Tangential section of a root grown in the absence of boron. Secondary root primordia are formed very close to the root tip.



SOMMER AND SOROKIN—ABSENCE OF BORON

SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 5. The four plants to the right were grown in the absence of boron for three weeks. They have very short stunted roots, secondary roots made very little growth. The three plants (controls) to the left were grown in the same kind of solution with the exception that boron had been added.

FIG. 6. Grown in the absence of magnesium and in the presence of boron.

FIG. 7. Grown in the absence of both magnesium and boron. The pathological changes in the tissues are typical for the absence of boron.

FIG. 8. Without sulphur. A slight irregularity may be observed in the periblem. The rows of cells are loosely connected.

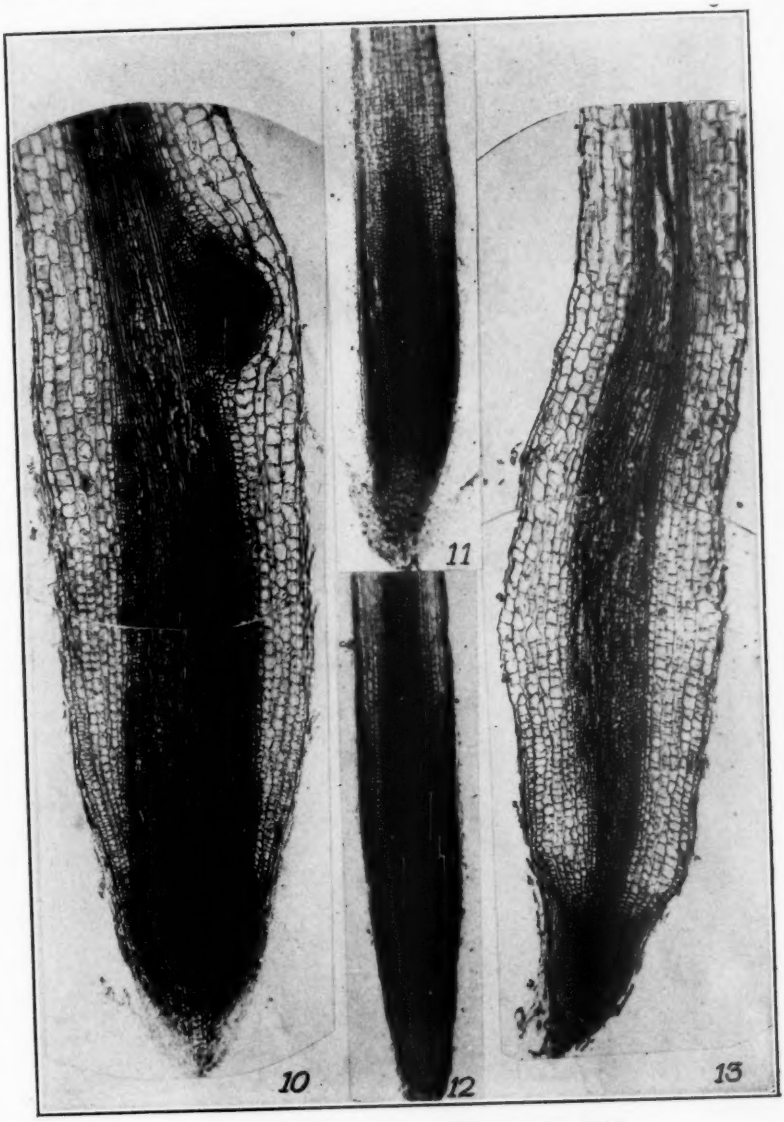
FIG. 9. Without sulphur and with no boron excepting a small amount occurring as an impurity in the salts. The rows of cells are very loosely connected. (A tangential section).

FIG. 10. Without manganese and without boron. Hypertrophy of the periblem and hyperplasia of the plerome are well developed. A branch root primordium may be seen in the upper part of the figure. Irregularly thickened cell walls are present in the periblem.

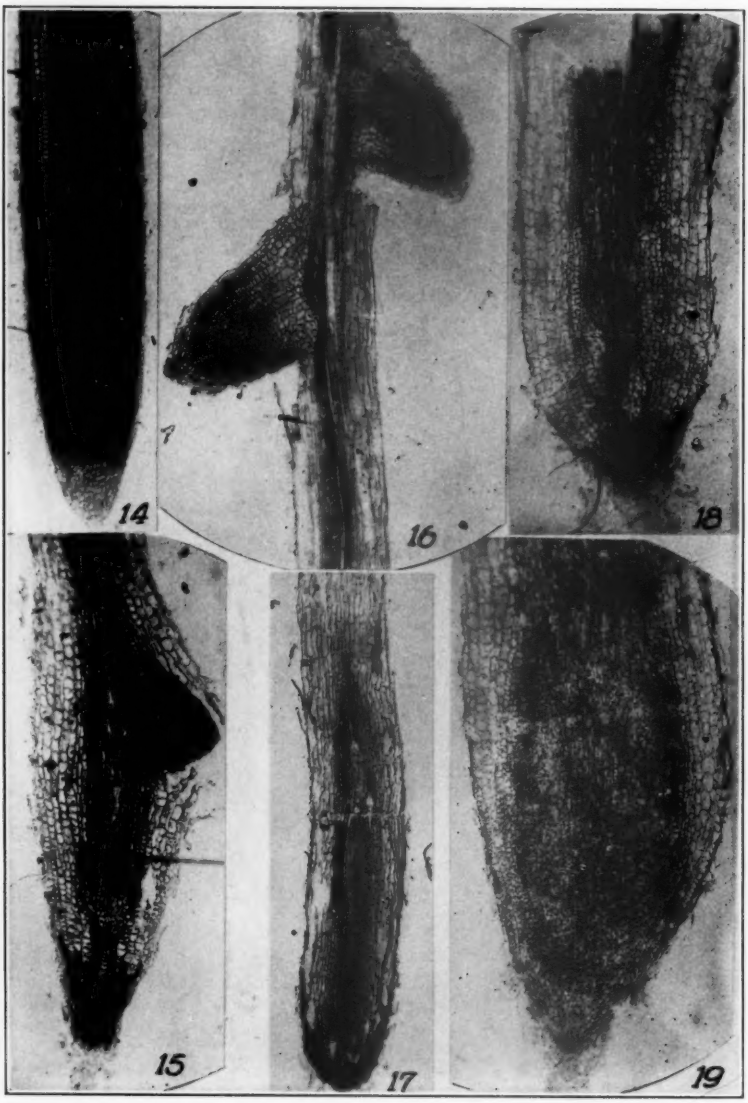
FIG. 11. Without manganese, with boron.

FIG. 12. Without potassium, with boron.

FIG. 13. Without potassium and without boron. Typical effects of the absence of boron are present.



SOMMER AND SOROKIN—ABSENCE OF BORON



SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 14. Without potassium and without boron. Grown for two weeks in pyrex containers. Pyrex yielded enough boron to supply the plant during the early stages of growth.

FIG. 15. As in fig. 14 but grown in pyrex for six weeks. The root shows some abnormality, but the typical effect of the absence of boron was not yet obtained.

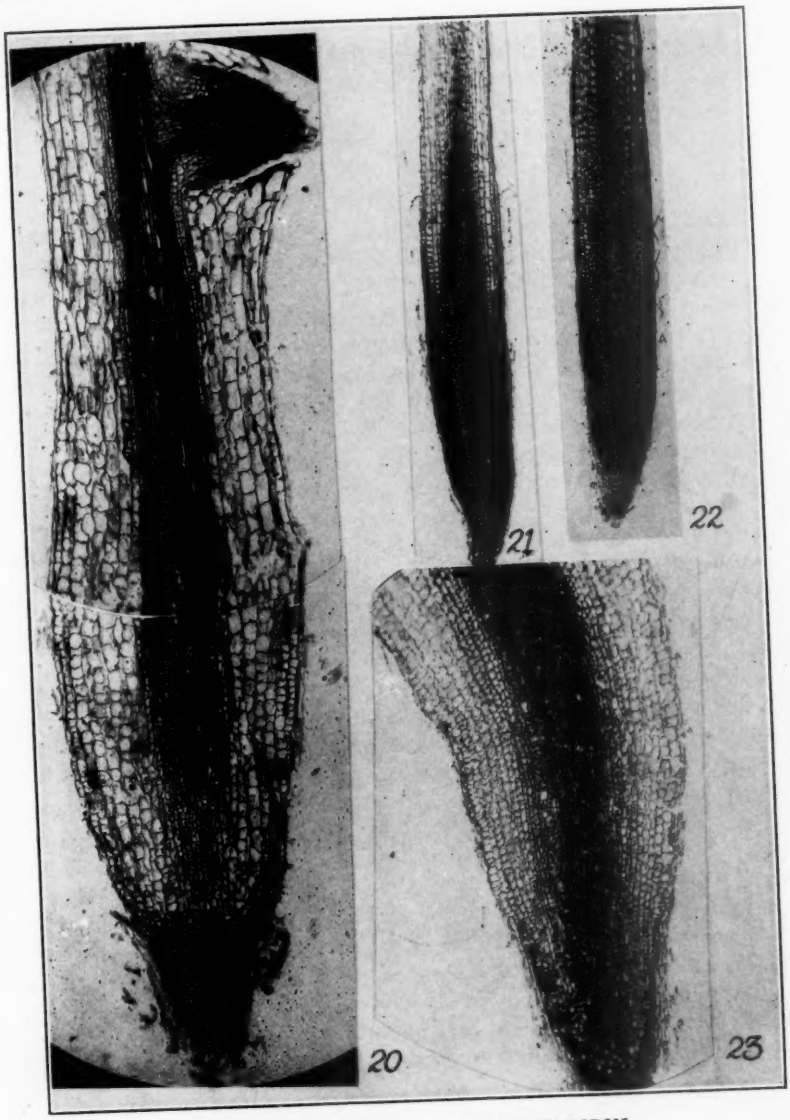
FIG. 16. Without nitrogen, with boron.

FIG. 17. Apical portion of root shown in fig. 16.

FIG. 18. Without nitrogen and without boron.

FIG. 19. As in fig. 18. Hyperplasia of the plerome is very well developed.

FIG. 20. Without iron and without boron. Typical effects of the absence of boron.
FIG. 21. Without iron, with boron.
FIG. 22. Without phosphorus, with boron.
FIG. 23. Without phosphorus and without boron.



SOMMER AND SOROKIN—ABSENCE OF BORON

CALCIUM, POTASSIUM, AND IRON BALANCE IN CERTAIN CROP PLANTS IN RELATION TO THEIR METABOLISM

WALTER F. LOEHWING

Introduction

Due to the widespread use of lime and potash in agricultural fertilizer practice, reports of instances in which these elements have proven injurious are continually increasing. Survey of the voluminous literature on responses of crop plants to mineral fertilizers reveals that injury following lime or potash amendments occurs most commonly when there is extreme lack of balance between these and other nutrient elements in the soil. Though lime and potash injury has been observed most frequently on mineral soils, highly organic soils often exhibit it even though acid in reaction or low in potash. Attempts to increase the fertility of poorly productive organic soils of the muck type in the thickly populated regions of the United States have not in general proven very successful on account of the peculiar physical and chemical characteristics of these soils.

Mucks are somewhat similar to peat soils in origin but their vegetable detritus has undergone such pronounced disintegration that the resulting particles, in contrast to the more bulky peat soils, betray surface phenomena to a remarkable degree. Conspicuous among these are adsorption and capillary effects which often become critical in determining the availability of nutrients and water. These unproductive mucks may superficially resemble productive loams by being dark in color and pulverulent in texture but they differ from them by being extremely light in weight, low in mineral content, high in combustible matter, high in water retentivity, and poor in thermal conductivity. Many of the singular responses of muck soils to fertilizers can undoubtedly be ascribed to their unique physical characteristics.

Earlier reports of fertilizer tests on organic soils showing injurious effects of lime and potash to crops (**7, 38, 39**) have not always included complete plant analyses and hence the exact physiological cause of the injury observed has not been understood. Some of these earlier tests (**11, 19**) covered a period of years in order that the effects of climatic fluctuations from year to year and the errors of immediate response observations might be avoided. The generally uniform behavior of the crops indicated that the injury could not be attributed to the climatic conditions of a single season. Chemical analyses accompanying more recent reports concerning injury of crops by lime and potash (**8, 12, 13**) show that the physiological cause underlying the injurious effect is frequently a disturbance in the

internal mineral nutrient balance so profound as to upset or prevent normal metabolism in the plant. Tissue analyses in such cases are far more valuable than soil analyses in indicating proper fertilizer practice to be pursued. The investigation herein described was undertaken to determine if chemical analyses of crop plants in their vegetative phase would indicate why the fertility of certain poorly productive, acid muck soils was apparently diminished by use of calcium and potassium fertilizers. For purposes of comparison, a soil similar to the above in composition and texture but responding favorably to lime and potash was included in the experiment.

Experimental procedure

Samples of soils known to exhibit marked response to lime and potash amendments were thoroughly mixed with finely powdered, chemically pure calcium carbonate or potassium chloride in the proportions indicated in accompanying tables (II, III, IV). 3200-gm. portions of each soil were placed in glazed earthenware pots and 800 cc. of freshly distilled water were added. Yellow Dent corn, Marquis wheat, and Mammoth Red Fancy clover were grown in duplicate pots on treated and untreated (check) soils in a greenhouse at 60° C. The weight of soil plus water in each pot was readily maintained at 4 kg. by daily replenishment of water lost in transpiration and evaporation, since neither was high. Plants were harvested for analysis at the end of the tenth week by removing entire pot contents to a large pan in which the roots were washed free of soil with distilled water. After rapid drying on filter-paper to remove excess water, the entire crop from each pot was weighed, minced and the pulp thoroughly mixed. Duplicate samples were retained for moisture determinations and the remainder of the pulp pickled for storage in boiling 80 per cent. alcohol containing 2 gm. calcium carbonate per liter. Preceding analysis, alcohol was evaporated and samples dried to constant weight in a vacuum oven at 80° C.

Four distinctly acid organic soils differing in their response to fertilizers were employed. The first was a black soil low in potash and light in weight responding favorably to lime but unfavorably to potash. The second soil was a brown, sandy, low lime muck, exhibiting injury following both lime and potash applications. The third was a black, light weight, powdery, low potash muck showing injury to cereals following use of lime but responding favorably to potash amendments. The fourth was a friable, black muck responding favorably to both lime and potash. Chemical analyses of the untreated soils are given in table I.

TABLE I

CHEMICAL ANALYSES OF FOUR UNTREATED MUCK SOILS

	PERCENTAGE DRY WEIGHT			
	SOIL 1	SOIL 2	SOIL 3	SOIL 4
Total calcium	1.67	0.08	3.44	3.54
Total magnesium	0.18	0.10	0.40	1.02
Total iron	0.10	2.02	1.21	2.22
Total potassium	0.30	1.52	0.29	1.05
Total phosphorus	0.34	0.08	0.19	0.20
Total sulphur	0.44	0.11	0.54	0.20
Total nitrogen	3.90	1.42	3.62	1.22
Volatile matter	83.00	42.61	48.10	47.78
Lime requirement, ppm.	2700.00	3500.00	2700.00	1200.00

Analytical methods

Lime requirements were determined by the VEITCH method and mineral content by official methods (**2**). Entire plants including roots were analyzed. Organic nitrogen was determined by the GUNNING method and was modified to include nitrates in determination of the total nitrogen content of tissues. Nitrate nitrogen was determined by difference. After clarification with neutral lead acetate, soluble sugars in an aliquot of alcoholic filtrate were hydrolyzed with concentrated hydrochloric acid and analyzed volumetrically for total reducing sugars by the modified BERTRAND permanganate method. Copper values are expressed as glucose determined from MUNSON-WALKER tables (**2**). Total carbohydrates include the polysaccharides of the dry residue (F_3 fraction) calculated as starch and determined by direct acid hydrolysis, plus the total soluble sugars of the filtrate (F_1 and F_2 fractions).

Moisture content is expressed as percentage of wet weight. Portions of original moisture samples were ignited and used for determination of minerals according to official methods. Calcium was precipitated as oxalate, potassium as cobaltinitrite, and iron as ferric hydroxide, the latter being subsequently reduced with zinc in concentrated sulphuric acid. All three were then titrated against standard potassium permanganate. Magnesium was determined gravimetrically as pyrophosphate. Cryoscopic methods were employed for the estimation of osmotic pressure of freshly expressed sap, the observed depression of freezing points being converted to atmospheric pressures (**16**). Hydrogen ion concentrations were measured potentiometrically.

TABLE II

CHEMICAL ANALYSES OF VICTORY OATS

Salt added (ppm.)	Percentage of dry weight								Sap extract		Dry yield (gm.)
	Iron	Magnesium	Calcium	Potassium	Nitrate nitrogen	Total nitrogen	Total sugars	Total carbohydrates	Osmotic pressure (atm.)	pH	
Soil 1											
Check	0.067	0.16	0.40	0.47	0.18	2.25	4.51	13.80	5.40	6.0	5.70
400 KCl	0.080	0.08	0.31	0.82	0.26	2.01	5.09	11.41	5.80	5.1	4.12
4000 $CaCO_3$	0.089	0.28	0.57	0.30	0.69	1.70	4.40	10.07	4.25	6.5	3.78
Soil 2											
Check	0.120	0.27	0.35	0.75	0.38	1.74	5.20	9.60	6.30	5.2	4.73
400 KCl	0.140	0.12	0.14	0.90	0.09	1.13	5.69	9.02	6.65	5.0	2.76
4000 $CaCO_3$	0.090	0.30	0.49	0.37	0.97	0.90	5.31	7.52	6.52	5.9	4.20
Soil 3											
Check	0.046	0.33	0.32	0.91	0.21	1.80	3.77	10.32	3.85	4.9	4.88
400 KCl	0.053	0.21	0.42	1.19	0.07	2.33	5.26	12.41	4.81	4.5	5.71
4000 $CaCO_3$	0.082	0.38	0.58	0.46	0.92	1.67	5.20	8.75	3.00	6.2	2.90
Soil 4											
Check	0.081	0.22	0.29	0.74	0.61	1.75	6.46	11.94	4.26	4.7	3.70
400 KCl	0.097	0.20	0.19	0.95	0.40	1.94	7.00	12.49	5.70	4.4	5.10
4000 $CaCO_3$	0.061	0.29	0.26	0.57	0.30	2.07	8.11	13.40	4.92	5.8	4.37

TABLE III

ANALYSES OF MARQUIS HARD WHEAT

SALT ADDED (ppm.)	PERCENTAGE OF DRY WEIGHT								SAP EXTRACT		DRY YIELD (gm.)
	IRON	MAGNESIUM	CALCIUM	POTASSIUM	NITRATE NITROGEN	TOTAL NITROGEN	TOTAL SUGARS	TOTAL CARBOHYDRATES	OSMOTIC PRESSURE (atm.)	pH	
Soil 1											
Check	0.021	0.24	0.72	0.28	0.20	2.27	6.85	12.93	7.24	4.6	5.78
400 KCl	0.028	0.20	0.39	0.70	0.47	2.07	6.90	9.66	10.13	4.2	4.48
4000 $CaCO_3$	0.026	0.24	0.50	0.20	0.80	1.40	4.90	10.14	9.90	6.0	3.39
Soil 2											
Check	0.140	0.36	0.41	0.74	0.85	0.99	5.24	14.58	8.87	4.6	5.80
400 KCl	0.145	0.28	0.27	0.90	0.29	0.64	4.47	8.77	9.00	4.7	3.20
4000 $CaCO_3$	0.117	0.39	0.65	0.44	0.70	0.90	4.97	10.40	9.22	6.0	5.10
Soil 3											
Check	0.014	0.17	0.32	0.78	0.10	0.76	6.88	14.21	7.26	4.9	5.47
400 KCl	0.029	0.20	0.24	0.89	0.00	0.94	5.98	13.60	7.40	4.5	6.72
4000 $CaCO_3$	0.009	0.31	0.38	0.54	0.56	0.70	3.94	9.95	6.05	5.7	4.28
Soil 4											
Check	0.121	0.36	0.38	0.67	0.74	0.79	4.44	13.46	7.27	5.0	4.87
400 KCl	0.165	0.32	0.18	0.82	0.40	1.35	6.12	14.80	9.03	4.7	3.96
4000 $CaCO_3$	0.042	0.40	0.51	0.48	0.71	1.30	6.78	14.05	10.03	5.8	4.80

TABLE IV

ANALYSES OF YELLOW DENT CORN

SALT ADDED (ppm.)	PERCENTAGE OF DRY WEIGHT								SAP EXTRACT		DRY YIELD (gm.)
	IRON	MAGNESIUM	CALCIUM	POTASSIUM	NITRATE NITROGEN	TOTAL NITROGEN	TOTAL SUGARS	TOTAL CARBOHYDRATES	OSMOTIC PRESSURE (atm.)	pH	
Soil 1											
Check	0.014	0.20	0.46	0.70	0.17	2.45	4.46	12.20	8.61	5.1	9.88
400 KCl	0.020	0.14	0.15	0.81	0.62	1.97	6.12	10.74	9.41	4.3	8.10
4000 $CaCO_3$	0.042	0.24	0.78	0.56	0.41	1.70	4.87	9.26	8.58	5.6	6.70
Soil 2											
Check	0.096	0.25	0.35	0.40	0.18	2.26	6.04	10.85	7.22	4.8	7.90
400 KCl	0.127	0.18	0.20	0.72	0.26	2.02	4.00	9.37	9.46	4.3	4.96
4000 $CaCO_3$	0.141	0.27	0.84	0.31	0.42	1.74	5.71	8.74	8.07	6.2	6.40
Soil 3											
Check	0.036	0.27	0.44	0.52	0.23	2.06	5.14	12.12	6.42	5.6	8.26
400 KCl	0.045	0.20	0.28	0.89	0.14	2.46	7.28	14.71	9.21	4.7	9.98
4000 $CaCO_3$	0.071	0.38	0.71	0.36	0.19	1.85	4.66	9.12	8.89	6.1	6.02
Soil 4											
Check	0.091	0.32	0.37	0.74	0.30	2.17	6.10	13.11	8.80	4.8	4.59
400 KCl	0.093	0.20	0.25	0.90	0.11	2.47	5.38	13.64	11.02	4.2	7.04
4000 $CaCO_3$	0.070	0.40	0.52	0.58	0.21	1.85	4.88	12.09	9.75	5.8	7.12

Discussion

The response of each of the three crops to a given fertilizer was quite consistently the same for each soil. High yields were as a rule associated with more rapid growth of the plants and generally improved appearance, involving more abundant and healthier foliage, stiffer stems, and greater stature. External evidences of injury, on the other hand, were stunted growth and procumbent or spindly habit associated with abnormal, often chlorotic or spotted leaves. Only on soils which had been treated with potassium chloride did plants develop stiff and erect stems, from which it may be inferred that abundance of potassium is more essential than lime for development of normal stem habit in grain crops grown on these soils. Microscopic examination of tissues from recumbent stems disclosed weak sclerenchyma cells with large lumina and poorly developed thickenings in tracheae, conditions suggesting disturbed carbohydrate metabolism especially with reference to polysaccharides.

The analytical data show that disturbed carbohydrate metabolism was coincident with reduced potassium content which, in the case of plants grown on the first three soils, became low enough to create a potash insufficiency, if yield and symptoms of injury be taken as criteria. That potassium is important in carbohydrate storage has long been known, though its precise mode of action is not yet clear. In an earlier report (**27**) it was noted that potassium may also be important in protein synthesis since the presence of abundant carbohydrates and inorganic nitrogen appear to favor protein accumulation (**35**). Accumulation of nitrates in high lime tissues may be due to the fact that lime increases soluble soil nitrates (**9, 32, 34**) which are absorbed by the plant but not converted to proteins because, potash being deficient, there are insufficient carbohydrates to combine with the large store of nitrates. Aqueous soil extracts analyzed for mineral nutrients indicated that there was soluble potassium present which presumably was available for absorption. The addition of alkaline fertilizers to soils is known to liberate other adsorbed bases and for this reason the quantity of potassium in the soil solution would be expected to increase rather than to diminish as a result of liming. Yet this is not necessarily the case for highly colloidal soils as deficient in minerals as those here considered. Many soil filtrates showed marked hysteresis, being initially acid to indicators but later becoming alkaline, a behavior not uncommon in extracts of organic soil (**36**). This phenomenon probably explains why duplicate analyses of organic soil solutions give inconcordant results and it probably also influences to a considerable extent the availability of plant nutrients in such soils. It is also necessary to point out in this connection that during absorption the selective permeability of root hairs comes into

play and the rate at which they take up soluble ions is not necessarily proportional to the concentrations of these in soils. Whatever may be the conditions controlling absorption of mineral nutrients from these soils, the fact remains that many plants on high lime soils showed external and internal symptoms of potash hunger apparently induced by the lime employed to correct acidity. Mature leaves were elongate, somewhat flaccid, dull in color with slightly wavy margins and puckered surfaces. Young leaves were distinctly yellowish. Some of these symptoms of potash insufficiency are similar to those previously observed in tobacco grown on sandy soils subjected to leaching (**12**).

The above described calcium-potassium balance of lime-injured plants does not fully account for the chlorotic condition of their young leaves, a symptom ordinarily associated with iron insufficiency. It was consequently at first difficult to reconcile the high iron content of these plants with their chlorotic appearance. The explanation of this apparent anomaly was suggested by the fact that the hydrogen-ion concentration of the sap of plants from limed soils was considerably lower than that of plants from unlimed soils, a condition apt to be of the utmost importance in controlling iron solubility (**3, 22, 37**) and absorption (**17, 30**). Microchemical tests for iron were made on tissues (**41**) to determine if reduced sap acidity interfered with iron translocation. These tests disclosed large amounts of iron in the nuclei of root hairs and the vascular elements of roots and basal stem nodes. The root hairs appeared healthy but there was evidence of obstruction by iron salts in many root tracheae and sieve tubes. Sections from higher stem nodes gave positive tests for iron in a few sieve tubes and companion cells while leaf sections, with the exception of a few from mature leaves, gave tests for traces or were entirely negative. High lime plants generally displayed this peculiar iron immobility, characterized by copious precipitation in roots with a tendency to diminish in aerial parts to such an extent that leaves displayed iron chlorosis. The chemical analyses here given (tables II, III, IV) disclosed no irregular distribution of iron because shoots and roots were not analyzed separately. Lime, then, not only created potash insufficiency but also reduced sap acidity to the point of interference with internal iron mobility.

Marsh and Shive (**28**) working with soy beans in solution cultures have pointed out that solubility of ferric salts, both in culture solutions and in sap, diminishes rapidly as neutrality is approached. Only when the sap is distinctly acid do adequate amounts of iron absorbed by roots actually reach the leaves. These investigators found maximum yields correlated with small quantities of iron evenly distributed in aerial parts of plants grown in moderately acid media. Availability of iron was correlated with

its solubility, and tolerance of the tissues to it was controlled by the nature of the culture solutions employed. Highly acid media were injurious due to iron toxicity. It is to be pointed out that the iron content of lime-injured tissues herein reported runs higher than that reported by MARSH and SHIVE. This is due to the fact that they rejected roots which were covered with precipitated iron and analyzed only tops which were low in iron as already explained.

There are also a number of other cases on record in which lime and the concomitant diminution of acidity reduced iron solubility to the detriment of plant growth. MAZÉ (**29**) induced chlorosis in corn and found that it might be due to deficiency of sulphates as well as iron in plants. Lime injury involving chlorosis has been reported for pineapples by GILE (**13**), for rice by GILE and CARRERO (**14**), for pears by MILAD (**31**), and for citrus fruits by LIPMAN (**26**). In spite of chlorotic conditions in the tops, accumulation of iron may sometimes become so great in roots and basal nodes as to be toxic, either directly or as a result of over-stimulating oxidase activity (**18**). HOPKINS and WANN (**20, 42**) find that iron is removed by adsorption on calcium phosphate which gradually precipitates as solutions become alkaline, a physico-chemical effect capable of influencing iron availability within the plant as well as in culture media. The effect of tissue colloids in controlling succulence, hardiness and imbibition is well understood and they are also undoubtedly important in nutrient intake (**24**). It has been noted by CHANCERL (**4**) and HANSTEEN-CRANNER (**15**) that calcium has a tendency to accelerate transpiration. In plants, a portion of whose tracheae have become obstructed by precipitation of iron, water loss may exceed the rate of replenishment from below, thus creating a marked saturation deficit in leaves and causing flaccidity. It will also be noted from the tables that osmotic pressures of sap were lower in plants on limed soils, indicating a general diminution of soluble materials as a consequence of calcium applications. In fact, it is possible that lime or alkalinity resulting from it interferes not only with the mobility of iron but with the mobility of other solutes as well.

The fact that lime applications proved beneficial on soil 4, in which iron and magnesium were relatively abundant, seemed attributable to the fact that iron toxicity was allayed and intake of iron reduced though not to the extent of inducing chlorosis, because leaf xylem and border parenchyma gave clearly positive microchemical tests for ferric ions. Untreated cultures of this soil produced plants with narrow, green leaves, speckled with brown and showing a tendency to turn yellow and die at the tips. This soil also responded favorably to potash amendments but, though crops improved in general vigor and in yield, small brown specks were still dis-

cernible in the leaves showing persistence of iron toxicity. Crops on this soil were benefited most by a combination of calcium carbonate and potassium chloride. Soil 4 was the least acid of those investigated, yet it is doubtful if acidity as determined by ordinary methods can be considered an index to the lime requirement of these or other highly colloidal soils (**43**, **44**).

Plants in the untreated cultures of soil 1 were stunted and produced small, smooth, flaccid, generally pale yellow, mottled leaves with green patches near the midrib, all of which symptoms were intensified by use of potash. Stems, however, were fairly stiff and erect. Even though sap acidity and iron content increased following use of potash, there was no evidence of iron toxicity, probably because the soil was too low in this element to permit excess absorption thereof. The symptoms of injury coupled with the results of plant analyses suggest that the explanation for the injurious effect of potash in the case of this soil lay in the fact that its increase in the tissues was made at expense of both lime and magnesium. In fact, it will be noted from the accompanying tables that there was a rather regular diminution of both calcium and magnesium in plants following use of potash, though magnesium hunger did not become apparent in all cases. To deficiency of calcium may be attributed the poor development of foliage, but coupled with this in producing the stunted condition of the plants may be disturbed root activity, since root hair production is known to be largely dependent upon an adequate supply of calcium (**5**, **10**, **40**).

KELLEY and CUMMINS (**23**) have reported low calcium concomitant with high potassium content of citrus leaves, in which mottling develops when calcium becomes insufficient. GARNER and others (**12**) have observed depression of lime and magnesium in tobacco, often to the degree of severe injury, following use of potash fertilizers. The characteristic symptoms of the injury called "sand drown" they found to be due to magnesium starvation. The tips of lower leaves first became chlorotic and from that point chlorophyll decomposition continued progressively until only the veins remained green. These investigators found use of potassium sulphate and heavy doses of potassium chloride responsible for the disease, at first thought due to heavy rains. Magnesium insufficiency, accentuated by use of potash, was apparently the reason for the unproductivity of soil 1, as it responded very favorably to applications of a mixture of dolomitic limestone with potassium chloride. As stated above, the unfavorable effect of lime alone on this soil was attributable to interference with internal mobility of iron.

On soil 2, potash injury was characterized by the chlorotic condition of young leaves which never became large nor very turgid. Older leaves

possessed a distinctly dull color and showed in addition to the characteristic yellow-green chlorophyll mosaic described above, numerous small brown specks indicative of excess iron. Soil 2 was relatively high in iron (table I) and crops grown on it showed a marked increase in absorption of iron following use of potash. A combination of dolomitic limestone and potash proved only slightly beneficial to this soil in spite of the fact that it was extremely low in magnesium, the failure to respond to this treatment being due apparently to the fact that iron remained toxic.

On soil 3 the response of crops to potash amendments was quite favorable. The potash-treated soils produced healthy, erect stemmed plants with large, deep green leaves, the potassium materially increasing the stiffness of the stem. Though increased potassium absorption resulted in somewhat smaller intake of lime and magnesia, the diminution in amount of these was not great enough to induce symptoms of calcium or magnesium hunger. The increase in soluble iron correlated with increased acidity was obviously beneficial to the plants because their green color was considerably intensified. Plants on this soil showed the greatest increase in osmotic pressure following potassium treatment, a response which was noted in the case of plants from the other soils but to a less marked degree. Potassium salts were found by GARNER and his collaborators (**12**) to increase the osmotic concentration of sap in tobacco, the rise in concentration being due to the greater concentration of electrolytes. When no potassium fertilizers were used, most of the osmotic components of tobacco sap were soluble organic constituents, probably carbohydrates.

The above described variations in mineral nutrient content were correlated in fairly definite ways with organic products in the plants investigated. Injury by lime or potash was in general marked not only by increases in nitrates but also by reductions in organic nitrogen and carbohydrates. High yields, on the other hand, showed generally reversed conditions. The fact that high-lime, low-potash plants show carbohydrate decrease coupled with nitrate increase may be taken to indicate that carbohydrates are insufficient to combine in proper amounts with inorganic nitrogen in protein formation. In these instances, lime applications have diminished potash content to the point of starvation which interferes with normal carbohydrate metabolism. The effects of potash starvation are accentuated by the disturbed iron mobility which induces chlorosis of tops in lime-injured plants. That is, lack of iron minimizes the photosynthetic area in leaves and lack of potash interferes with carbohydrate storage. If potassium is essential for the synthesis of carbohydrates and inorganic nitrogen into proteins, its deficiency in lime-injured plants is another reason for their low organic nitrogen content.

Potash injury, though resulting in reduction of carbohydrate and organic nitrogen content, operated in an indirect manner, apparently by increasing sap acidity which often favored iron toxicity. In the case of soils 1 and 2, magnesium absorption was so depressed that symptoms of its insufficiency also became apparent, and in these instances magnesium chlorosis was associated with excess soluble iron in interfering with photosynthesis. Consequently, the ultimate result of injury by potash or lime was reduction in the amount of carbohydrates and organic nitrogen. High yields were in general correlated with more nearly balanced conditions among mineral nutrients, and with high carbohydrate and protein content.

The great variability in concentration of lime and potash in the plants grown on these muck soils is in part attributed to the highly colloidal nature of the soil particles, which on the one hand prevent leaching of nutrients but on the other may create physiological deficiency thereof by adsorption. Injury from a given fertilizer to plants on colloidal soils may be direct or it may be due to toxic substances liberated by replacement. Mineral fertilizers added to organic soils may depress the solubility of elements present only in small quantities and thereby make them unavailable to plants. For these reasons chemical analyses of unproductive muck soils give little clue to the proper fertilizer practice to be pursued in improving them. In such instances plant analyses indicate in a more satisfactory way the mineral nutrients required, at least for the early vegetative phase of the grain crops here reported. Fertilizers needed during maturation stages would probably be different from those required in early development due to the differences in metabolism during vegetative and reproductive phases in the same species (**6, 25, 33**).

Summary

Grain crops on acid muck soils low in potash are often injured by additions of calcium carbonate, which may entail potash hunger and consequent interference with carbohydrate storage, or induce chlorosis by making sap too alkaline to maintain iron in solution. Additions of potassium chloride to such soils may prove injurious to grain plants by increasing iron accumulation in the tissue to the extent of toxicity, or by reducing lime and magnesium content to the point of starvation. Sap is more acid in high potash than in high lime tissues. Soil and sap acidity favor iron solubility and absorption. High yield in young grain plants was associated with high carbohydrates and high organic nitrogen. Low yields were characterized by low protein, low carbohydrate, and high nitrate content.

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AN APPARATUS FOR THE GROWTH OF PLANTS IN A CONTROLLED ENVIRONMENT[1]

A. R. Davis and D. R. Hoagland

(with six figures)

Introduction

The vagaries of an uncontrolled environment are responsible for many of the conflicting results and conclusions found in publications dealing with plant growth. It is common knowledge that workers in different institutions, and indeed often in the same institution, find great difficulty in duplicating the findings of one another, not on account of inadequate description of methods, but because some environmental influence was not controlled. This is particularly true where yield is the criterion upon which the interpretation is based. We have in mind as an example a comparison of the growth obtained in two diverse types of culture solutions.[2] Wheat grown in them under natural conditions of illumination and temperature during various months of the year gave yields significantly the same during the winter months and significantly different during the summer period. One's conclusions in such a case would obviously depend upon the time of year the observations were made. Again in a more recent investigation, the data for which are given in table I, similar results are apparent.

TABLE I

The influence of seasonal solar illumination on yield of Little Club wheat. Temperature 20.5° C.

Period	Nov. 22/26 Dec. 20/26	Dec. 29/26 Jan. 27/27	Feb. 18/27 Mar. 18/27	Mar. 25/27 Apr. 22/27	May 2/27 May 30/27	Aug. 17/27 Sept. 13/27
	cgm.	cgm.	cgm.	cgm.	cgm.	cgm.
Mean dry weight 100 tops	25.7	15.2	39.9	46.2	97.5	86.9

In this last experiment the same lot of pure line seed was used throughout and germination conditions, temperature, culture solutions, etc., were maintained as nearly identical as possible in the several series. The chief

[1] The authors wish to acknowledge the generous grants made by the University Board of Research, without which this work could not have been pursued.

[2] Unpublished data.

variable was illumination and it was sufficient to cause a fluctuation in yield from 15 cgms. in December to 97 cgms. in May! When an environment is characterized by such conditions of change the duplication of yields becomes a chance achievement and conclusions drawn from them may frequently be just as much a matter of guesswork. Such data as these indicate the necessity of conducting plant experimentation, whenever possible, under controlled conditions. The mere recording of climatic fluctuations is insufficient because of the difficulty of isolating the effect of a single variable.

The construction and maintenance costs of equipment rather than obstinate mechanical problems or a failure to realize the importance of this method of attack has prevented most plant physiologists from taking the steps necessary toward control of environment. One has in mind more particularly the extensive equipment in use at the Boyce Thompson Institute (**1, 2**). Such equipment is highly to be desired, permitting as it does the study of plant growth over a wide range of conditions adequately controlled by dependable instruments. It would be most unfortunate, however, if failure to finance a similar installation should discourage the worker who has a limited budget at his disposal. There are many problems which may be attacked just as efficiently with a small and relatively inexpensive control equipment such as the one employed by TOTTINGHAM (**10**) at the University of Wisconsin, that of HOTTES at the University of Illinois, or the one we wish to describe in this paper.

We have had in mind an apparatus that would permit efficient control of the environment, would not require a large maintenance fund, and at the same time would meet requirements for expansion both with respect to budget and research program. The apparatus now in use in our laboratory is the result of several years experience and while still in a state of development, it is meeting the demands placed upon it in a highly satisfactory manner. Moreover it contains, we feel, certain essential elements of design which will make an appeal to workers attacking diverse physiological problems in botany and in the allied fields of plant pathology, horticulture, agronomy, etc. It must be borne in mind that this apparatus is not designed for extensive growth investigations where large numbers of plants are involved and where soil is employed as a culture medium. It is rather for the intensive study of plants easily handled by the water culture method. The latter makes for economy of space and permits control of the source of inorganic ions. The equipment is peculiarly adapted to the study of nutrient solution effects in a controlled environment; studies on the influence of light intensity and exposure time; light-temperature relationships; the effect of differentials in temperature between roots and tops;

humidity effects; changes in the gaseous components of the atmosphere; factors influencing the production of organic compounds, etc., etc. These are but a few examples of the many problems which will at once occur to the reader. With wheat as the plant under investigation it has been possible to grow as many as 100 plants for a period of four weeks in each chamber and as is indicated in table II, this will give a mean of unusual reliability.

There are two important tests of such an apparatus. First, the plant grown must compare favorably in appearance and composition with that obtained under favorable conditions in nature, and second, there must be the possibility of reproducing environments and as a consequence replicating results. We have found our apparatus extremely satisfying in both these respects. As far as we have been able to determine by superficial appearance and by chemical analyses, illumination has been satisfactory both as to intensity and quality. The wheat plants have been green, vigorous, have tillered well, and have shown etiolation only when illumination was deliberately decreased. These points are illustrated photographically in fig. 1, where the two cultures on the left, grown under artificial light in

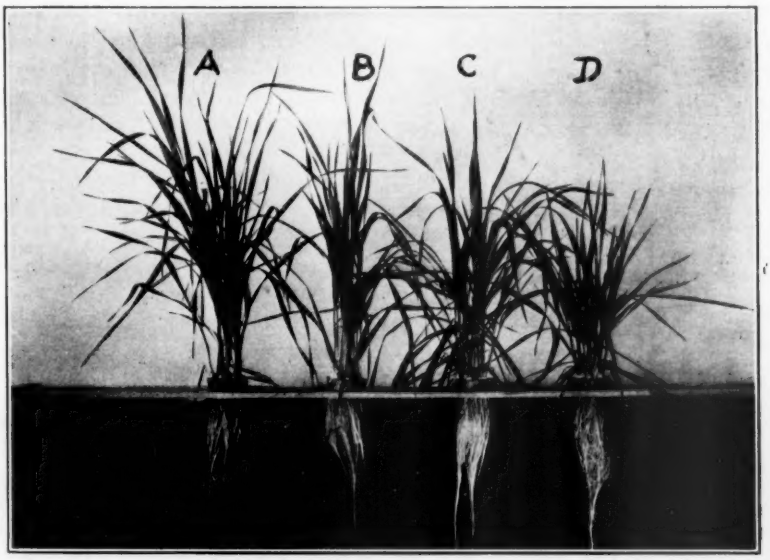

FIG. 1. Comparison of the effect of artificial and solar illumination on growth of wheat during March. A = 2610, B = 1640 foot candles electric light, 16 hours per day at 20.5° C. C and D, full solar illumination. C = 20.5° C. and D = temperature uncontrolled. Plants 28 days from transplanting.

a controlled environment may be compared with those on the right grown at the same temperature under solar illumination during March. A still more striking comparison is shown in fig. 2, where the culture on the left was subjected to solar illumination during December at 20.5° C., the one in the center and on the right to artificial light, the first at 17° C., the second at 20.5° C.[3]

As a general statement we may say that wheat plants grown in a controlled environment at 2600 foot candles artificial illumination, 16 hours daily exposure time, and at 20.5° C. exceeded in yield those of parallel series grown in a greenhouse under solar illumination and at the same controlled temperature for any month of the year with the exception of May. During this month natural light conditions were especially favorable and approximately equivalent yields were obtained. The above statements have reference to 28-day plants only. However, we have demonstrated to our own satisfaction that although considerably hastened, normal maturation of the plant can be effected in such a controlled environment. The two mature Little Club wheat plants shown in fig. 3 show normal tillering, heading and seed formation, the heads being above average for field conditions.[4] The plants averaged seven heads, each maturing 50–60 well filled kernels, these latter being 30 per cent. heavier than the parent seed. The increased weight was probably due to a higher nitrogen content. Previous investigators have reported the maturation of wheat under continuous artificial illumination, HARVEY (**2**), MAXIMOW (**6**), and BAKHUYZEN (**11, 12**) among others. HARVEY reports the development of a single head with "6–8 well filled seeds" where the illumination was continuous and of 400–800 foot

[3] The plants shown in figs. 1 and 2 were grown for 28 days in two quart Mason fruit jars containing 1850 cc. of solution. Five plants were grown in each jar. The solution employed was one which has been used in this laboratory over a period of years and for many different plants. The elements are supplied as $Ca(NO_3)_2$, KNO_3, KH_2PO_4, $MgSO_4$, HBO_3 and $MnSO_4$ in the following parts per million concentration:

Ion	NO_3	PO_4	SO_4	K	Ca	Mg	B	Mn
Ppm.	700	10	200	190	172	52	0.5	0.5

The plants grew rapidly and the iron requirements were relatively high. PO_4, added from time to time, was maintained at the above low level in order to facilitate iron absorption, otherwise there was no addition of elements during the period noted. K and NO_3, the two ions most rapidly removed, were present in amounts sufficient for the growth period under the conditions imposed. Fe was added as ferric tartrate, 2 cc. of a 5 per cent. solution per jar as needed.

[4] According to Prof. W. W. MACKIE, Division of Agronomy, University of California.

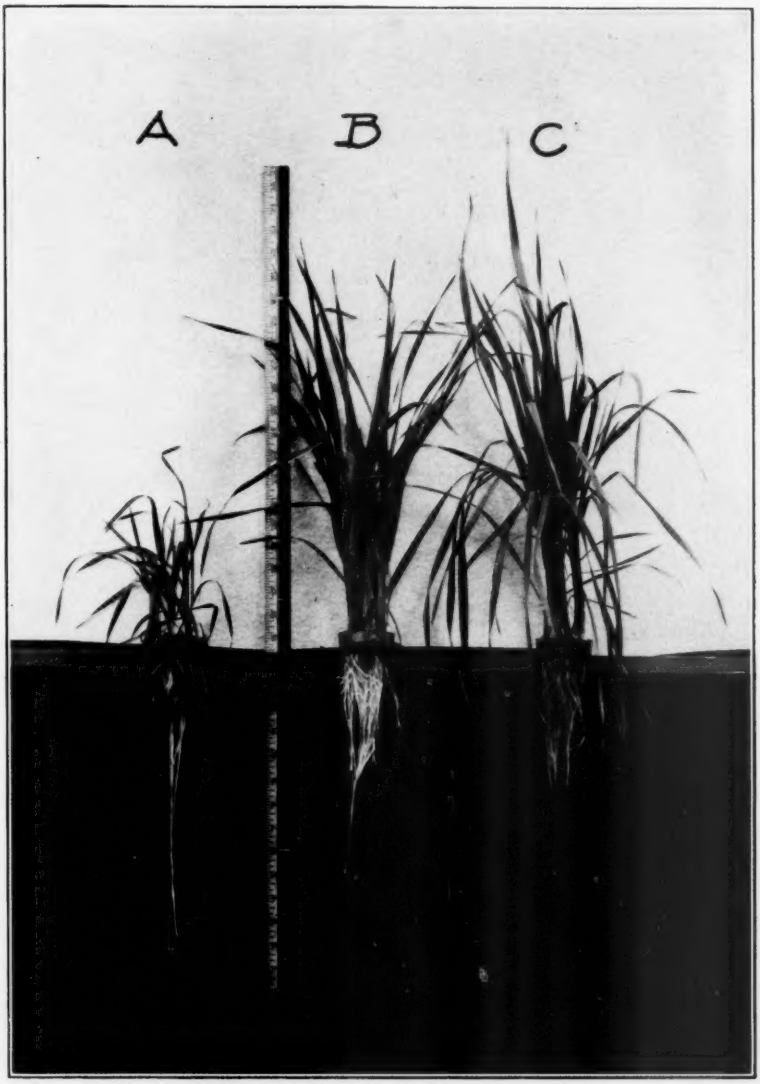

FIG. 2. Comparison of the effect of artificial and solar illumination on the growth of wheat during December. A = full solar illumination 20.5° C. B and C = 2610 foot candles electric light, 16 hours per day, B at 17° C. and C at 20.5° C. Plants 28 days from time of transplanting.

FIG. 3. Two plants Little Club wheat 13 weeks from time of planting. Artificial light = 2610 foot candles, 16 hours per day, 20.5° C.

candles intensity. MAXIMOW does not state the number of heads and seeds formed, nor mentions the appearance of the seeds. BAKHUYZEN has grown Hard Federation to maturity under continuous electric light, one stalk only being permitted to develop. The head and seed formations were stated to be normal.[5]

Duplication of yields has likewise been achieved with a high degree of satisfaction as is apparent from the data in table II. Here the mean dry weight of 100 tops shows a remarkable uniformity and the probable error of the mean (approximately one per cent.) is unusually low. Calculations based upon this probable error indicate less than a 30:1 chance that the greatest differences involved are significant. These data and others to be reported in a subsequent paper, strikingly indicate the feasibility of controlling the environment and of predicting results. Such predictions are not only possible with respect to height and weight of plants at the end of a predetermined period, the time of first tiller appearance, the final number of tillers, etc., but in addition an equation can be developed enabling

TABLE II[6]

DUPLICATION OF WHEAT YIELDS IN A CONTROLLED ENVIRONMENT ILLUMINATION, 3600 WATTS (2610 f. c.) 16 HOURS DAILY EXPOSURE PERIOD. TEMPERATURE, 20.5° C. DURATION OF EXPERIMENT, 28 DAYS

EXPERIMENTAL PERIOD	NUMBER OF PLANTS	MEAN DRY WEIGHT TOPS	MEAN NUMBER TILLERS	HEIGHT MAIN STALK
		cgm.		cm.
Dec. 29, 1926 } Jan. 26, 1927 }	100	92.2 ± 0.94	4.0	59
Feb. 18, 1927 } Mar. 18, 1927 }	100	90.5 ± 0.90	4.0	60
Mar. 25, 1927 } Apr. 22, 1927 }	100	89.0 ± 1.00	4.2	57
Oct. 27, 1927 } Nov. 24, 1927 }	100	89.6 ± 0.95	3.9	59
Oct. 27, 1927 } Nov. 24, 1927 }	100	90.6 ± 0.90	3.8	58

[5] Several earlier workers have demonstrated the feasibility of substituting artificial for solar illumination. For an historical account of this work the reader is referred to POPP'S (7) excellent summary.

[6] In these series a pure line strain of Little Club wheat obtained through the courtesy of Professor W. W. MACKIE, of the Division of Agronomy, this University, was used. The seeds were soaked in the above culture solution for 16 hours at 20.5° C., then distributed on tinned wire netting over pyrex baking dishes containg the same solution. The atmosphere above the seeds was kept saturated with water vapor until the roots were well in contact with the culture medium. At this point the cover was removed and the seedlings grown under 600 watts artificial light until they attained an average height of 9 ± 5 cm. Only those falling within these limits were selected for transplanting.

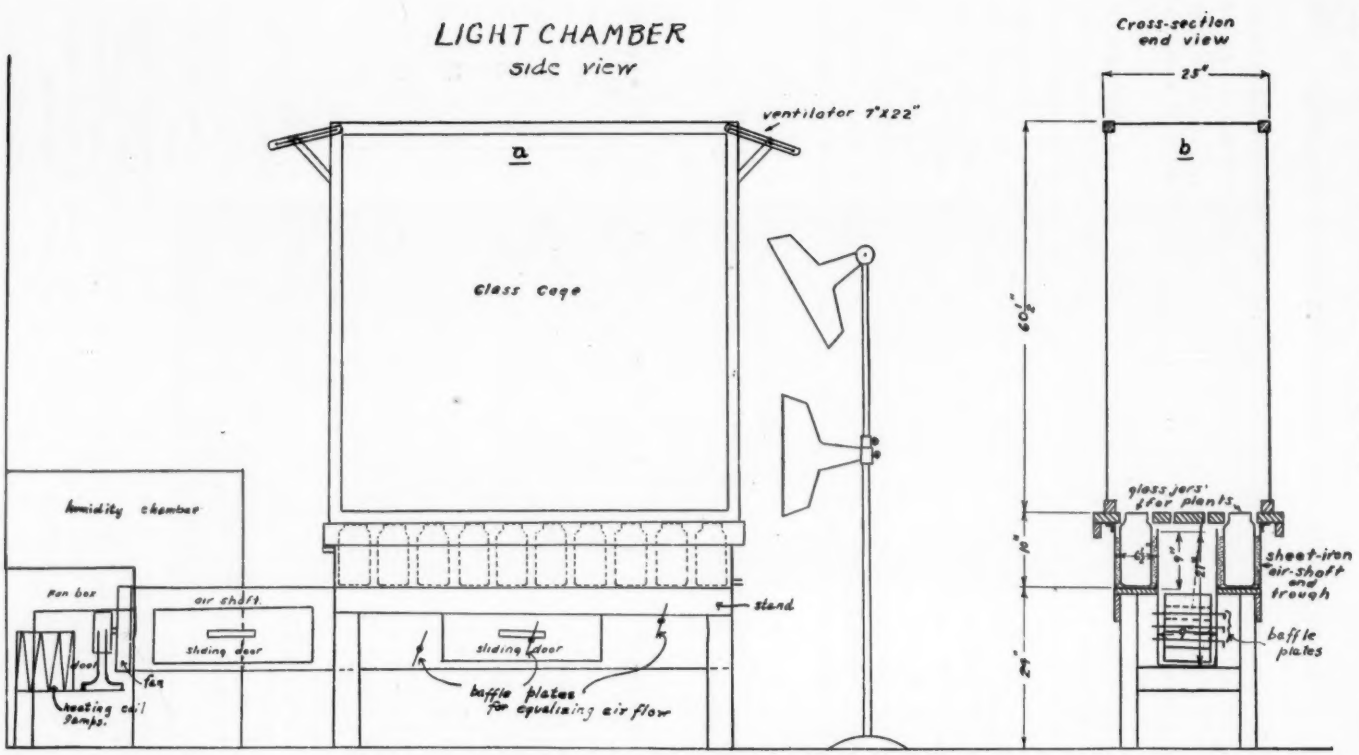

FIG. 4. Apparatus for the control of environment. Side view.

one to predict yields for varying daily exposure times where the intensity of light remains constant. Thus with all the major influences in the environment controlled, one is in a position to study one plant intensively and to learn much of the part played by each portion of the environment at each stage of growth. Moreover, such control and duplication of results enables one to definitely establish a "standard plant" as a reference point.[7]

Apparatus

The apparatus is constructed in units, two of which have been in operation during the past year and a half (figs. 4, 5, 6). Each unit is housed in a white enamelled room, 12 x 12 x 12 feet, provided with bottom ventilators as shown in fig. 5. In addition to these latter, a large opening in the

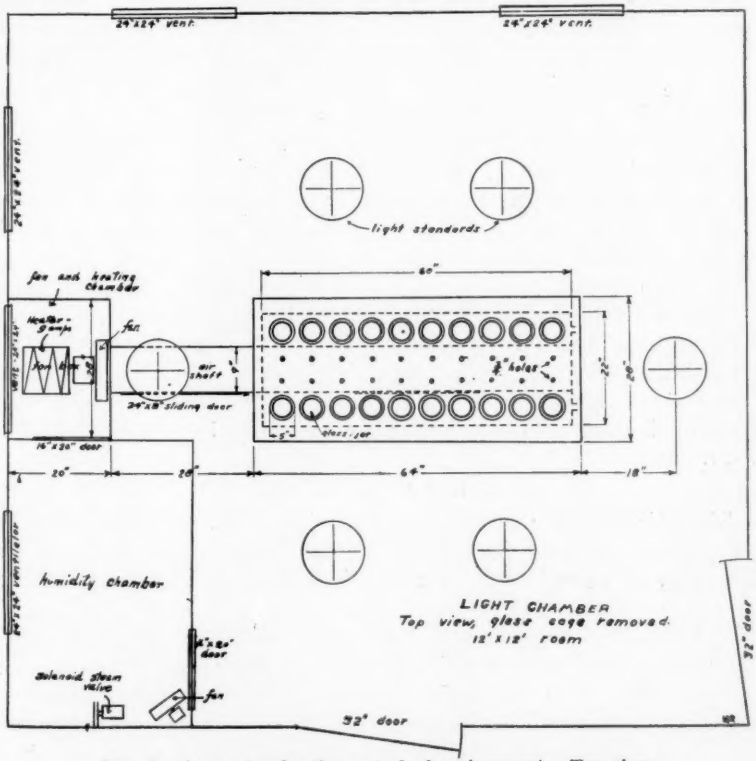

FIG. 5. Apparatus for the control of environment. Top view.

[7] The desirability of plant experimentation under these conditions has been frequently emphasized and is generally admitted. Among others, TREALEASE and LIVINGSTON (9) had this in view in their report on the relation of climatic conditions to the salt-proportion requirements of plants, and again LIVINGSTON (5) in his survey of recent advancements in plant physiology.

ceiling of the room permits an excellent natural draft which may be increased by the use of electric fans. Exclusive of lighting, heating, and humidity control, which will be described separately, the apparatus consists of a plant chamber and its accessories as follows: (a). perforated wooden platform upon which the glass chamber rests; (b). temperature and humidity chamber housing fans, heating and humidity apparatus; (c). air tunnel leading from the temperature-humidity chamber to the plant chamber; (d). galvanized iron troughs upon which the wooden platform rests and which holds the culture solution jars.

Plant chamber

The plant chamber in which the cultures are placed (fig. 6) is of high grade glass (not plate) 5 x 5 x 2 feet, open at the bottom where it rests on the wooden platform and counterpoised over pulleys in the ceiling of the room to permit raising with a minimum of effort. The wooden platform resting on the galvanized iron troughs and air tunnel is pierced with twenty 5-inch holes for culture jars as well as a number of 1-inch holes to facilitate the flow of air from the air tunnel through the plant chamber (figs. 4b, 5). The air tunnel, occupying a space between the troughs and below the platform, leads to the temperature chamber where is placed a 12-inch electric fan and an electric heating unit (figs. 4a, 5). Air flows along the air tunnel, through the perforations in the wooden platform into the plant chamber, thence out through vents at the top of the latter (fig. 4a). The rate of flow through these vents as measured by a "Tycos" anemometer has been established at 400 cubic feet per minute, the equivalent of a gentle but constant breeze over the plants. To equalize air flow in different portions of the plant chamber, adjustable baffle plates are placed in the air tunnel (figs. 4a–b).

Temperature control

The desired temperature is maintained within 1° C. by means of a double relay system controlled by a DeKhotinsky bimetallic thermostat.[8] The first relay, a telegraphic 100 ohm type, is activated by the current from a six volt wet battery; the second relay, a General Electric Co. magnetic switch[9] with twenty-ampere capacity on the secondary, is controlled by the 110 volt A. C. flowing through the secondary of relay no. 1. We have found the two relay system very satisfactory where the amperage required for heating is high since a small current may be sent through the thermostat and first relay and no trouble experienced because of sticking of contact points on the former. As an additional precaution a telephone condenser

[8] Central Scientific Co. No. 13740.

[9] Gen. Elec. C. R. 2610–1265–G 1–1102–60 cyc.

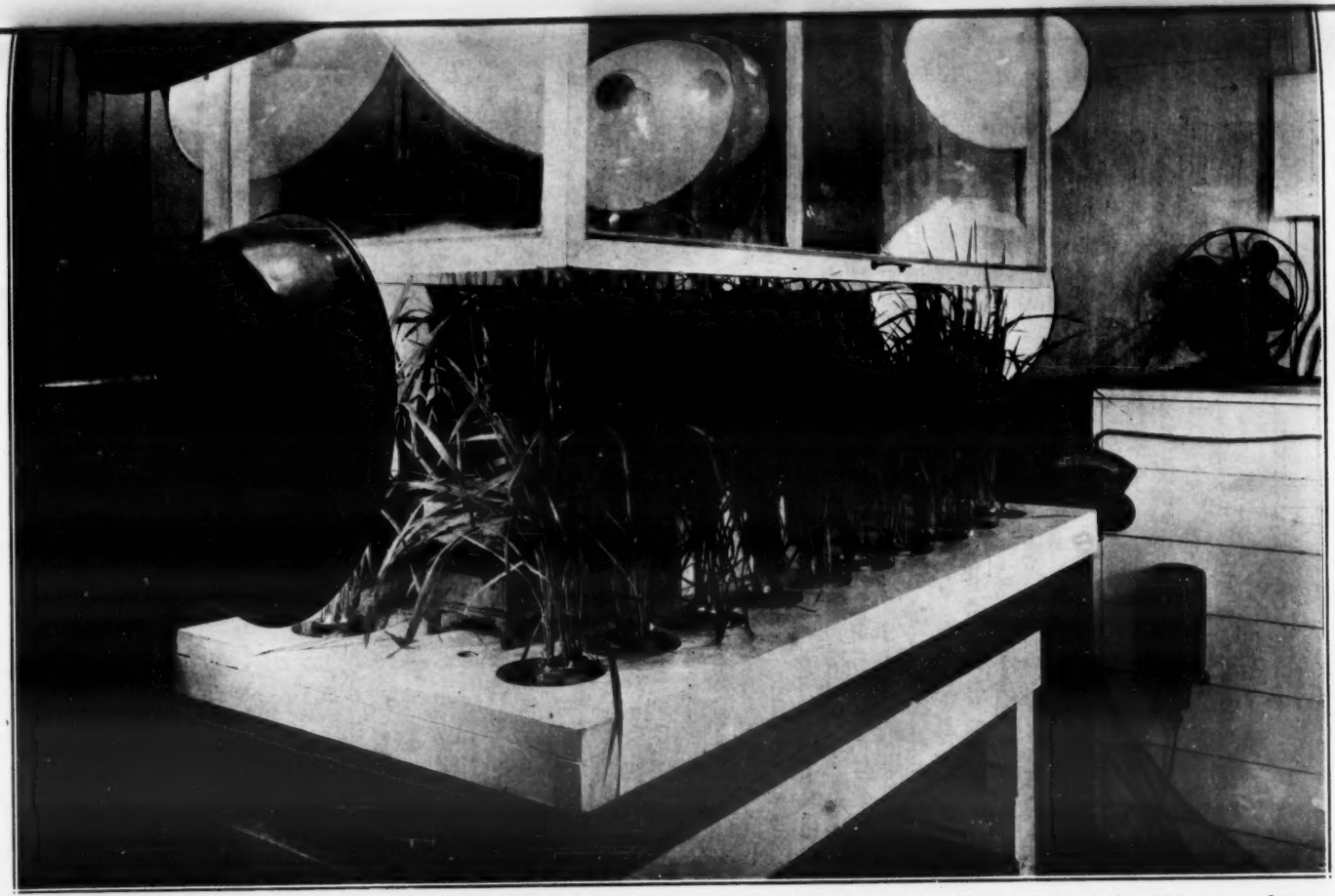

FIG. 6. Apparatus for the control of environment showing 28-day plants grown at 3035 foot candles, illumination intensity, 16 hours per day, 20.5° C. Counterpoised plant chamber partly raised.

is placed across the primary circuit. The heating unit used is homemade and consists of a transite frame 10 x 10 x 10 inches wound with enough no. 24 "Chromel" wire to give it a capacity of 9 amperes. During the cooler months the heat from the lamps is employed as a secondary heating source and by restricting ventilation in the room all or only part of such heat may be utilized. These sources of heat meet the requirements at Berkeley. It is realized however, that in colder sections of the country it may be desirable to pre-heat the air by means of steam or hot water pipes and employ an electric heating unit to give final control. In case a large plant chamber is used and air replacement is relatively slow, steam heat may be used directly, the temperature being regulated by means of a Bristol or similar type controller as is the practice at the Boyce Thompson Institute.

Provision has been made for the maintenance of a differential in temperature between roots and tops, should such be desired. The troughs (fig. 4b) acting as supports for the culture jars are fitted with inlets and outlets so that water of any predetermined temperature can be circulated. This feature precludes the use of a rotating table as employed by TOTTINGHAM (**10**) and by HARVEY (**3**). However, owing to the rather rigid balancing of conditions in different parts of the chamber, we feel such a mechanism is not necessary, although as a precaution against any undetected environmental inequalities, it has been the practice to change the position of the jars at regular intervals. The unusually small probable error of the mean (noted earlier) as well as the uniformity of distribution on a frequency curve indicate no unusual asymmetric influences.

In installations of this sort the disposal of heat from lamps having a maximum emission in the near infra-red presents a problem, the solution of which usually involves the interposing of a water screen between lamps and plants. Although efficient from the point of view of heat absorption, certain secondary difficulties are encountered which tend to decrease the value of the method. The glass water screen must be kept scrupulously clean to prevent a lowering of light intensity in the visible spectrum. Furthermore, unless the correct temperature relation is maintained between glass and air beneath, moisture is condensed on the lower surface of the former. This method, with its pumps and water circuit adds to the complexity of the equipment and limits one in the distribution of lamps. It was found possible to simplify our original apparatus in which we employed a water screen, by substituting a slow current of air through the plant chamber and a relatively rapid replacement of air in the surrounding room. In the present rooms the excellent natural draft is usually all that is required to keep the plant chamber at a point within two degrees of air at the intake. A refrigeration system would add greatly to the range of experimentation.

Illumination[10]

The method of heat dissipation described permits the placement of lamps above and on all four sides of the plant chamber as well as at any distance from it.[11] The lamps are on movable standards, adjustable as to height, and are equipped with a heavy cast iron base. The standards, two lamps to each, are distributed two on each side of the plant chamber and in addition two lamps are suspended from the ceiling above. In the experiments cited the filament of all lamps was within ten inches of the glass wall and approximately sixteen inches from the growing plant. The distance of the upper lamp from the plant changed slightly with growth but not enough to significantly affect the illumination values given. The reflectors employed are the dome type[12] eighteen inches in diameter and possess a wide angle of dispersion. The manufacturer's rating gives a reflecting efficiency of 76 per cent.

In all the experiments reported herein unless otherwise noted, we have made use of the 300 watt Mazda C gas-filled lamp, the energy distribution being very similar to that given by Popp (8) for a 1000 watt lamp of the same type. Although these lamps are rated at 1000 hours their efficiency under our conditions began to fall off noticeably after 500 hours both with respect to total energy and energy in the visible spectrum.[13] This latter decrease is more marked because the maximum energy band, which in these lamps lies in the short infra red, shifts still further in the direction of longer wave length with use. This is due to volatilization of the filament resulting in increased resistance, lower consumption of current and decreased temperature. Because of such deterioration it has been our practice to install new lamps at the beginning of each four week period so that illumination values might be easily duplicated. Where lamps of one experiment were carried over to another, yields lower than anticipated were obtained. This obviously should be expected.

[10] A Hartford time switch of 220 volt-50 ampere capacity is employed for controlling the daily illumination period (Hartford Time Switch Co., 71 Murray St., N. Y.)

[11] It is realized that some light is lost into the surrounding room by this placement of lamps. Theoretically it might be more efficient to have the side walls of the plant chamber of some highly reflecting material and all lamps overhead. The slight loss in light efficiency with our arrangement, granting sufficient intensity for the purpose at hand, is offset by the opportunity given to observe plants and instruments through the glass walls.

[12] Gen. Elec. Co. R. L. M. Standard Dome 500.

[13] The Gen. Elec. Co. performance curve indicates a 7 per cent. decrease in candle power for this period and for 120 per cent. of the rated life of the lamp, a loss of 20 per cent. Harvey (3) on one occasion used similar lamps a total of 3000 hours. At this time they were undoubtedly producing less than fifty per cent. of their rated output in foot candles.

Intensity determinations are made at present with a single junction, iron-nickle thermocouple in circuit with a galvanometer sensitive to 10^{-7} amperes. Such an instrument must be used with caution since it measures total energy output rather than that of the visible spectrum. By means of suitable wave filters the instrument can be adapted to determination of energy distribution and by calibration against a standard lamp visible radiation may be recorded in terms of candle power or as ergs per cm.^{2} per second. The thermocouple is quite sensitive to direction of radiation. It is our practice therefore to place it within a six liter frosted flask, the integration of radiation from different angles thus being made possible. The set up is placed at different positions within the plant chamber and equalization of illumination is easily achieved by adjustment of the lamp standards.[14]

Humidity

Control of humidity is still in the development stage. Although the Bristol system[15] of control has been installed our conditions have not been altogether satisfactory for its successful operation. The apparatus consists of a sensitive nitrogen filled bulb operating in conjunction with a "controller," a relay and a solenoid steam valve. A change in wet bulb temperature as induced by humidity fluctuations, makes or breaks a weak electric circuit controlling the steam valve. Steam injected into the chamber is the humidifying agent. It is planned to construct a large underground chamber as a reservoir of humidified pre-heated air.

The lack of humidity control has not been a serious one in our studies so far. The moisture content of the air at Berkeley is remarkably uniform throughout the year and the fluctuations which have been met have not influenced yields as may be seen by reference to the dry weight column in table II.

[14] The 300 watt Mazda C lamp has a lumen output of 17.7 per watt or a total of 5300 lumens, 12.57 lumens equaling one spherical foot candle. The reflector efficiency is 76 per cent. thus $\frac{5300 \times 76}{100 \times 12.57} = 320.5$ foot candles per lamp. The average of a number of galvanometer readings for a lamp one foot distant was 35 mm., the thermocouple being housed within the frosted flask. With the housed thermocouple in the plant chamber one foot above the perforated platform and at the temperature of the preceding measurement, the galvanometer reading was $\frac{320.5 \times 285}{35} = 2610$ foot candles in the plant chamber. This was the intensity employed for the yields given in table II and is considerably higher than that given for the Boyce Thompson Institute light room where 25000 watts were employed, giving an intensity of 400 foot candles (8). Harvey (4) lists intensities ranging from 50–10,000 foot candles with the usual range lying between 300–2000 foot candles.

[15] The Bristol Co., Waterbury, Conn.

Cost of equipment

The total cost of the control apparatus including plant chamber and its accessories, light standards, reflectors, fans, temperature and humidity control, but not the thermo-hygrograph, was approximately $500.00 a unit. The plant chambers were constructed in the University shops and the source of the other equipment has been noted in the text. Electric current is the main item of maintenance expense and the amount of this will obviously depend on the nature of the investigation and the local cost of current. For the conditions noted in table II and with current at 3 cents per K.W., the cost per day per unit for lighting, heating and fans was approximately $2.00. The cost of lamps under the same conditions will range from fifty to seventy-five cents per day, depending upon size and frequency of replacement.

Summary

1. Equipment is described which permits the growth of plants under controlled conditions of light, temperature, humidity, and culture solution.

2. The apparatus is constructed in units, thus making possible easy expansion, with enlargement of research program and budget. The initial cost of the unit described was approximately $500, and the cost of maintenance per day of use approximately $2.50, with electricity at three cents per K. W.

3. The data given definitely show the feasibility of establishing an environment which can be duplicated at will, thus leading in turn to the duplication of yields, number of tillers, height of tops, and other external evidences of growth. Such a controlled environment permits the isolation of a single variable and a quantitative study of diverse physiological phenomena.

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University of California.

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[16] Since the submission of proof of this manuscript the authors have received a reprint from Prof. H. Lundegårdh of Stockholm on the "Photosynthesis of cultivated plants, its relation to yield and its dependence on climate and soil." (Meddelande N:r 331 från Centralanstalten för försöksväsendet på jordbruksområdet. Avdelningen för lantbruksbotanik N:r 43.) The article is in Swedish but has an excellent summary in English. Lundegårdh describes an apparatus in which plants are grown under controlled light, temperature, humidity, and carbon dioxide. The photographs indicate smaller units than the one herein described. Light is all from above and heat from the lamps is taken care of by a water screen. There seem to be many features about the apparatus that merit attention.

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ALUMINUM TOXICITY[1]

FORMAN T. MCLEAN AND BASIL E. GILBERT

Introduction

This subject has been the occasion for some controversy and misunderstanding among different workers. It has been asserted by a number of workers (**3, 5, 7, 8, 10, 11, 13, 15, 16, 18, 19**) that aluminum salts are decidedly toxic, and that the toxicity of acid soils is due primarily to this cause (**1, 2, 6, 7, 9, 15**). Others maintain that aluminum compounds are so insoluble that they cannot occur in solution in a sufficient concentration to cause injury to plant growth (**4, 12, 13**). Investigations of soils from widely scattered localities indicate that active aluminum is most abundant in leached-out acid soils of humid regions such as Hawaii, the Pacific Northwest, and the Eastern states of the United States.

As was shown in a previous paper (**16**), plants vary in their susceptibility to aluminum poisoning. Lettuce, beets and some other plants are very sensitive, while corn is more resistant and redtop is notably so. The injury caused to sensitive plants is localized mainly in the roots, which are dwarfed and inhibited from branching. The aluminum accumulates in the protoplasm and particularly in the nuclei of the cortex of roots immersed in a nutrient solution containing aluminum. Such accumulation has been noted in plants sensitive to aluminum but not in the roots of resistant plants, even when subjected to high aluminum concentrations. Aluminum poisoning causes the affected plants to absorb dyes, water, and nutrient salts less readily than normal plants.

Aluminum stimulation

Like some other toxic elements, aluminum is stimulating to plants in dilute concentrations. STOKLASA (**21, 22**), MAZÉ (**14**), and others (**20**) have found small quantities to be beneficial.

SERIES I

Cultures were grown in nutrient solutions with distilled water, using the same technique as reported in a previous paper (**16**). The plants were grown in 250-cc. jars, six plants to each jar, and solutions were changed twice weekly, supplying the phosphate and the aluminum at alternate changes. The acidity of the cultures was adjusted to approximately the same pH in all of the cultures in any one series by additions of Na_2CO_3 or

[1] Contribution no. 365 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

of H_2SO_4, so that the cultures containing aluminum were no more acid than the controls. The results are given in table I.

TABLE I

EFFECT OF DILUTE CONCENTRATIONS OF ALUMINUM SALTS ON THE YIELD OF VARIOUS PLANTS GROWN IN DISTILLED WATER SOLUTION CULTURES

AIR-DRY WEIGHTS PER PLANT

CONCENTRATION OF ALUMINUM	Al AS SULPHATE		Al AS CITRATE				
	RYE	OATS	ALFALFA	BUCKWHEAT	OATS	ONIONS	REDTOP
ppm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.0	0.46	1.18	0.190	0.66	1.50	0.17	0.51
3.4	0.86*	.....	0.069	0.72	2.26	0.20	0.42
6.8	0.66	1.31	0.264*	0.79*	1.82	0.16	0.22
13.6	0.40	1.23	0.075	0.66	2.58*	0.39*	0.72*
27.2	.....	0.86	0.018	0.39	2.36	0.24	0.49

* Optimum growth.

While these results are based on very few plants, six in each culture, and the results are somewhat irregular, the magnitude of the differences are so great that more replications would not be expected to change the main results. They clearly show stimulation by concentrations of 3.4 to 13.6 parts per million of aluminum added to what is ordinarily considered a complete nutrient solution. This table, however, does not include any of the plants which have been found to be markedly sensitive to aluminum poisoning.

Solubility of organic and inorganic aluminum compounds

Most workers have found that inorganic compounds of aluminum, sulphate, and chloride are easily precipitated, and remain in solution only in decidedly acid media, pH 4.5 or less. By alternating the applications of phosphorus salts and aluminum salts at successive changes of the culture solutions, it has been easy to maintain strongly toxic concentrations in solutions held at a pH of 4.0 to 4.5. The solutions containing aluminum were strongly toxic while non-aluminum solutions of approximately the same acidity were not seriously injurious to any of the plants tested.

Aluminum in combination with organic acids was found to be less easily precipitated. In the absence of phosphorus salts in the solution, aluminum citrate remained in solution even in nearly-neutral solutions. This was substantiated by a dialysis experiment, the results of which are given in table II. Twenty-five cc. each of nutrient solutions containing all of the usual salts and in addition 0.24N aluminum citrate, were placed in three dialysis

thimbles impregnated with pyroxylin, and each immersed in 200 cc. of distilled water for seven days. The acidity of the solutions placed inside the thimbles was pH 6.5.

TABLE II

DIFFUSION OF ALUMINUM OUT OF PYROXYLIN THIMBLES INTO DISTILLED WATER AFTER SEVEN DAYS

EXPERIMENTS	AMOUNT OF Al INSIDE THIMBLE		AMOUNT OF Al OUTSIDE THIMBLE	
	gm.	ppm.	gm.	ppm.
Thimble 1	0.0063	250	0.0355	178
Thimble 2	0.0068	271	0.0358	179
Thimble 3	0.0066	263	0.0345	172

Five times as much aluminum diffused out through the pyroxylin membranes as remained behind in the thimbles, thus showing that aluminum citrate diffuses readily at pH 6.5, and is in true solution, not colloidal.

Phosphorus and aluminum

Aluminum is so readily precipitated as aluminum phosphate that this factor has been eliminated in the foregoing experiment (table I), by supplying the plants with aluminum- and phosphorus-containing solutions at alternate changes of the culture solutions. Thus the plants were supplied with aluminum during half of each week and with phosphorus during the other half.

SERIES II

By this procedure the phosphorus and aluminum were supplied separately and the aluminum could not interfere with the absorption of phosphorus by precipitating it from solution outside the plant. However, it has been found that large amounts of phosphate fertilizer supplied to the soil diminished or prevented aluminum toxicity, and it was thought that possibly a larger amount supplied to plants in solution culture might increase their phosphorus content and decrease their susceptibility to aluminum poisoning. This was accordingly tested by supplying plants in 250-cc. glass bottles with two different levels of calcium acid phosphate, alternating with aluminum sulphate. The results are given in table III.

The additional amount of calcium acid phosphate seemed to depress the yields of rye and corn but to affect the yield of lettuce very little. Further, the higher amount of phosphorus did not noticeably reduce the aluminum toxicity except in the case of the highest aluminum dosage in corn and, in this case, the depression in both series of cultures was so severe that the

TABLE III

EFFECT ON ALUMINUM TOXICITY OF DIFFERENT LEVELS OF PHOSPHORUS SUPPLIED TO PLANTS IN SOLUTION

Rye			Corn		Lettuce		
PO_4 supplied			PO_4 supplied		PO_4 supplied		
Al added	0.00096N	0.00288N	0.00096N	0.00288N	Al added	0.0096N	0.00288N
	Weight of control plants					Weight of control plants	
	gm. 3.46	gm. 1.70	gm. 5.10	gm. 4.04		gm. 5.20	gm. 5.55
	Relative weight of plants					Relative weight of plants	
ppm.	per cent.	per cent.	per cent.	per cent.	ppm.	per cent.	per cent.
0.0	100	100	100	100	0.0	100	100
3.4	92	92	.....	.....	1.0	77	54
6.8	69	72	.....	.....	1.7	90	74
13.6	74	79	96	93	2.3	83	70
27.2	64	65	77	18	3.2	.....	79
40.8	64	64	59	51	4.1	80	73
54.4	58	50	12	25	.....	.....	.....

difference between the depression to 12 per cent. and to 25 per cent. yield, respectively, may not be significant. The tap water used in growing these cultures contained approximately 3 to 4 parts per million of aluminum, and was the same for all of the cultures in this series.

Series III

To test the effect of aluminum and phosphorus in the nutrient solution together, cultures of barley were started in February, 1927, and grown to maturity in quart Mason jars, 6 plants to each jar, in complete nutrient solutions (**17**), containing both phosphorus and aluminum in the same solution. Further, the solutions were not changed during the course of the experiment, and the cultures had a pH of 4.0 to 4.5 at the beginning, becoming about 5.9 toward the end of the growth period. One-half of the full nutrient supply and one-half of the amounts of aluminum salts intended for each culture were put in at the beginning, and the remainder was supplied at the sixth week, when the plants were half grown. Six plants were placed in each culture, and each culture triplicated with the exception of the last three. One culture not triplicated received extra phosphorus. The yields are summarized in table IV.

The added phosphorus, when it exceeded the equivalent of the added aluminum, proved beneficial when added with the aluminum. This is shown by the yield of culture 15 which exceeded that of the culture receiving no

TABLE IV

YIELDS OF BARLEY FROM SOLUTION CULTURES WITH DIFFERENT AMOUNTS OF PHOSPHORUS AND ALUMINUM (14 AND 27 PPM. RESPECTIVELY)

CULTURE NOS.	1, 2, 3,	4, 5, 6,	10, 11, 12,	13, 14,	15
Treatment per plant	milli-equivalents		milli-equivalents		milli-equivalents
PO_4	0.15	0.15	0.15	0.39	0.63
Al	0.0	0.27	0.54	0.54	0.54
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.
Tops	2.40	1.30	2.00	1.70	2.80
Roots	0.40	0.30	0.25	0.40	0.50

aluminum and with the lower amount of phosphorus. Phosphorus was intentionally made suboptimum in all of this series of cultures.

SERIES IV

Since phosphorus counteracts aluminum toxicity when they are placed in solution together, it would appear that its beneficial action occurred by precipitating the aluminum as aluminum phosphate. But is colloidal aluminum toxic to plants when in contact with the roots? It has been assumed by MAGISTAD (**13**) and others that aluminum is toxic only when in crystalloid form. This was tested by a special arrangement of three cultures grown at the same time as the preceding set. The plants were all grown during the first six weeks in pint jars with nutrient solution like cultures 1, 2, and 3 of the last series. Then their roots were confined in collodion sacks exactly the size of pint jars, and into these sacks were poured the residual solutions from the first six weeks of growth. These sacks containing the plants were suspended in quart jars with the added nutrients for the remainder of the growth period, but omitting the phosphorus. Then to culture 7 the phosphorus was added outside the collodion membrane. Culture 8 received both phosphorus and 1.62 milli-equivalents of aluminum sulphate outside the membrane so that any aluminum or phosphorus would have to pass through the membrane to reach the roots. Culture 9 received the same amounts of aluminum and phosphorus as culture 8, but poured inside the membrane and thus placed within reach of the barley roots which by this time extended to all parts of the space enclosed by the collodion sack.

Culture 9, the roots of which were in contact with the precipitated aluminum, promptly developed typical symptoms of aluminum toxicity, becoming discolored, and ceasing to grow normally. The other two cultures maintained normal roots. The yields of these three cultures are shown in table V.

TABLE V

YIELDS OF BARLEY CULTURES TO SHOW WHETHER COLLOIDAL ALUMINUM IS TOXIC

CULTURAL CONDITIONS	YIELD PER PLANT		P REMAINING	
	ROOTS	TOPS	INSIDE	OUTSIDE
	gm.	gm.	ppm.	ppm.
Culture 7, in collodion sack, nutrients outside, no Al	0.3	1.0	.....	.....
Culture 8, in collodion sack, nutrients + Al + P outside	0.2	1.6	0.03	0.02
Culture 9, in collodion sack, nutrients outside, Al + P inside	0.2	0.7	0.09	0.13
Culture 5, no collodion sack, nutrients + Al + P	—	—	0.06	—

Thus the aluminum was apparently unable to pass through the collodion membrane, for culture 8 showed no evidence of aluminum toxicity. Culture 9, however, subjected to presumably the same sort of aluminum compounds as were present outside the membrane in culture 8, was severely injured, indicating that colloidal aluminum can still cause toxicity to barley when placed in contact with the roots. If this conclusion is accepted then colloidal aluminum may be toxic to plants growing in soil likewise.

SERIES V

Another series of barley plants was grown to maturity in tall 3-liter biological specimen jars, with more liberal phosphorus supply, to test the effects of added aluminum sulphate and citrate on the yield. Culture solutions were changed and nutrients and aluminum were added weekly. According to the plan developed by PEMBER (17), the amounts of the nutrients were varied with the age of the plants. The cultures were divided into two series; cultures 1 to 12 were to test the toxicity of aluminum sulphate and were maintained quite acid at pH 4.6, while cultures 13 to 24 were to test the toxicity of aluminum citrate. Since aluminum citrate has been found not to precipitate out of solution even when neutralized, these latter cultures were maintained at pH 6.0. The even numbered cultures 14 to 24 inclusive, were grown with distilled water, while the others were grown with well water. The nutrient solution contained calcium nitrate, potassium chloride, magnesium sulphate and ferric nitrate. The results are shown in table VI.

Aluminum, either sulphate or citrate, was toxic with the addition of 16 parts per million, and about equally so either with the lesser or greater amount of phosphorus. In the distilled water cultures, there being no alu-

TABLE VI

YIELDS OF BARLEY IN SOLUTION CULTURES SUPPLIED WEEKLY WITH VARYING AMOUNTS OF NUTRIENTS, BUT WITH UNIFORM AMOUNTS OF ALUMINUM AS SULPHATE OR CITRATE (5 AND 16 PPM. RESPECTIVELY)

ALUMINUM SULPHATE

CULTURE NOS.	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12
Treatment per plant	milli-equivalents			milli-equivalents		
PO_4	0.216	0.216	0.216	0.648	0.648	0.648 Alternating
Al	0.0	0.201	0.603	0.0	0.201	0.603
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.
Straw	1.8	1.2	0.5	1.3	1.7	0.7
Grain	0.5	0.5	0.1	0.6	0.6	0.1

ALUMINUM CITRATE

CULTURE NOS.	14	16	18	20	22	24
Treatment per plant	milli-equivalents			milli-equivalents		
PO_4	0.216	0.216	0.216	0.648	0.648	0.648 Alternating
Al	0.0	0.201	0.603	0.0	0.603	0.300
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.
Straw	1.3	1.1	0.8	1.3	0.9	1.7
Grain	0.6	0.3	0.1	0.5	0.2	0.7

CULTURES WITH DISTILLED WATER

CULTURE NOS.	13	15	17	19	21	23
Treatment per plant	milli-equivalents			milli-equivalents		
PO_4	0.216	0.216	0.216	0.648	0.648	0.648 Alternating
Al	0.0	0.201	0.603	0.0	0.603	0.300
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.
Straw	0.80	0.8	0.80	0.4	1.2	1.0
Grain	0.05	0.3	0.03	0.1	0.2	0.1

minum in the basal solution, 5 parts per million of aluminum was more beneficial than none at all, again showing aluminum stimulation; and 16 parts per million was evidently deleterious to the grain yield.

Summary

A number of different kinds of plants was grown in solution cultures to study their susceptibility to injury by aluminum and to test the efficiency

of the phosphate radical as a preventive of aluminum injury. Very low concentrations of aluminum, 3 to 13 parts per million, were found to be stimulating to plants, while higher concentrations were toxic. Aluminum in combination with organic acids, such as citric and tartaric, was found to be dialyzable and to be toxic at very low acidities, pH 6.5 or even less acid. So high acidity is not an invariable or necessary accompaniment of aluminum toxicity. Further, non-dialyzing forms of aluminum which could not pass through a collodion membrane in sufficient amounts to cause toxicity, were decidedly toxic when placed in contact with the roots of barley.

Phosphorus, when put into solution with aluminum compounds, completely overcame the toxicity of the aluminum when the concentration of the phosphorus in the form of phosphate was equivalent to that of the aluminum. This was presumably brought about by the precipitation of the aluminum as aluminum phosphate, as additional amounts of phosphate, in excess of an amount needed for nutrition, exerted no beneficial effect whatever when it was supplied alternately with the aluminum at successive changes of the culture solution, so that no precipitation of aluminum phosphate could take place in the solution outside of the plants.

Conclusions

1. Soluble phosphate, in concentrations equivalent to that of aluminum, completely counteracts aluminum toxicity.

2. Aluminum, even when in the medium in non-diffusible, colloidal form can induce toxicity when in contact with roots of barley.

3. Aluminum as citrate is toxic even in solutions with very low acidity, pH 6+.

4. Aluminum is stimulating to plants at low concentrations, and toxic at higher ones.

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SOME STUDIES ON THE HARDINESS OF CERTAIN SPECIES OF *VACCINIUM*[1]

W. G. Brierley and A. C. Hildreth[2]

For the past twelve years the Minnesota Agricultural Experiment Station has been studying the possibilities of bringing the blueberry (*Vaccinium pennsylvanicum* Lam., *V. canadense* Kalm., and *V. corymbosum* L.) under field culture. The work has been hampered at times by winter injury to the plants. Such injury has occurred nearly every year with *V. corymbosum*, the common high bush blueberry of the east, and has been met with more or less frequently in the two native species. This recurring injury has led to a study of the hardiness of these species.

Observations in the field have shown that the most severe injury in the case of *V. corymbosum* has occurred in late fall. Under conditions prevailing at the Forest Experiment Station, Cloquet, where the field work is carried on, this species usually grows late, failing to mature its wood before the advent of severe freezing weather. Usually early snows have served to protect the lower portions of the new shoots, but the upper portions exposed to temperatures no lower than $-10°$ C. generally have been killed. Injury of this sort probably is due to lack of maturity and consequent lack of hardiness, rather than to inability to endure such temperatures in seasons which permit normal maturity and hardening off.

It appears likely that the lack of maturity in the shoots of *V. corymbosum* at Cloquet may be due in part to a short growing season. Blossoming and the beginning of shoot growth in this and the native species does not occur until late in May. Frosts in May or early June frequently are the cause of light crops through injury to the blossoms. Frosts at this time of year also are usually accompanied by low daily maximum temperatures under which shoot growth takes place slowly if at all. Light frosts frequently occur in late August and killing frosts are common in early September. Under such fall conditions shoots of *V. corymbosum* are frequently exposed to killing frosts before the formation of winter buds at the tips of the shoots has progressed very far, and before the normal maturing of the foliage has occurred. This is the condition of shoots described as poorly matured or showing a lack of maturity. Full maturity in the shoots of *V.*

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[2] The authors wish to express their appreciation of the assistance and cooperation in these studies of Prof. T. S. Hanson, Assistant Superintendent, Forest Experiment Station, Cloquet, Minn.

corymbosum as evidenced by well-developed buds at the tips and by maturity of foliage has occurred only once (1926) during the twelve seasons this species has been grown at Cloquet.

The native low bush species *V. pennsylvanicum* and *V. canadense* occur over a more northerly range than the *V. corymbosum*. It was thought, therefore, that they might be greatly superior in hardiness to the latter and could be used as a source of hardy parents in the production of hybrids with the *V. corymbosum*, which, because of its height and bush-form is a more desirable horticultural type. However, in the spring of 1924 extensive winter injury was noted in these native species, both in the cultivated plots and in the wild. In this season there was no snow cover until mid-January and sub-zero (F.) weather had been frequent. Again in the spring of 1925 similar injury was noted. This winter also was characterized by low temperatures in December with little snow. The question was raised therefore, whether the native species are materially hardier than *V. corymbosum* or whether they merely escape injury most years by reason of their low growth habit which enables them to be protected by a minimum snow cover.

Studies to determine the relative cold resistance of matured shoots of the three species were begun in the fall of 1925. Controlled temperatures under artificial refrigeration were employed, the freezing apparatus and methods being the same as used by HILDRETH (3) in determining the relative hardiness of apple varieties. Samples of new shoots and older wood were collected at Cloquet on October 22 after exposure in the field to a minimum temperature of $-10.5°$ C. At this time the new shoots of *V. corymbosum* were showing the characteristic injury resulting from freezing while immature. A few exceptionally vigorous shoots of *V. pennsylvanicum* also showed this type of injury although not to the same extent as *V. corymbosum*. Through a misunderstanding this material was subjected to a minimum temperature of $-12°$ C. in the freezing chamber, only 1.5° C. lower than the minimum to which they had previously been exposed in the field. After standing for some time with the butts in water in a cool greenhouse the samples showed no injury except as observed in the field.

Shoots of *V. pennsylvanicum* and *V. corymbosum* collected at Cloquet on January 26, 1926, were placed in a cool greenhouse with the butts in water. As the buds pushed into growth it was evident that there was only a little more injury at the tips than was found in the samples collected in October. It should be noted that although the air temperature had fallen as low as $-32°$ C. in December, the plants of *V. pennsylvanicum* were protected by snow and were probably not exposed to such a low temperature. The snow did not entirely cover the plants of *V. corymbosum*, but the exposed tips had already been killed in October as previously mentioned. Evidently the

snow had been sufficient to protect these plants against serious additional injury.

The value of snow protection for blueberries has been pointed out by COVILLE (**2**). He notes that in February, 1918, shoots of low bush hybrids were killed at Whitesbog, New Jersey, at a temperature of about – 12° F. while at Greenfield, New Hampshire, the parent stock, when protected by snow was uninjured although here the temperature dropped to – 30° F. The same season at Crotched Mountain, N. H., he found that tops of high bush blueberries, which projected above the snow, were killed back while the bases and sides of the same bushes which had been protected by snow bore the usual crop of berries.

Summarizing the results for the winter of 1925–1926, it appears that killing in the three species studied was due largely to a combination of immaturity plus early cold. The most severe injury occurred in October at temperatures no lower than – 10.5° C. *V. corymbosum,* which probably was not well matured, suffered most. Winter cold caused very little additional injury as the plants presumably were protected by snow.

In the fall of 1926 material was again taken for freezing tests, collection being made at Cloquet, October 21. Most of the new shoots were matured to the tip buds. Only a few of the very vigorous and immature shoots of *V. corymbosum* and *V. pennsylvanicum* showed slight freezing at the tips. Temperature records at Cloquet during the months of September and October showed a gradual decline, a minimum of – 6° C. having been experienced up to the time the material was collected.

Such fall weather conditions are generally considered to favor the development of hardiness. WINKLER (**5**) has shown that a gradually declining temperature increases the cold resistance in woody plants. REIN (**4**) and others have found that northern plants are able to accommodate themselves to cold, the plants becoming more resistant by exposure to temperatures that are relatively low but still above the lethal point. Under the conditions which prevailed at Cloquet this fall, it is assumed that full maturity was reached and that these species had attained about their maximum hardiness for the time of year.

After collection the samples were wrapped in damp moss and kept cool to hold them in condition till freezing tests could be made. On October 23 one lot of shoots from each species was exposed for three hours to a temperature of – 16° C. A second lot was similarly exposed on the following day to – 20° C. and a third lot on the 25th to – 24.5° C. All lots after freezing were stored in damp moss until October 26th when they were placed with the butts in water in a cool greenhouse. Three weeks later the shoots were examined and compared with control samples to determine the extent of

injury from the three treatments. The results obtained are shown in the accompanying table I.

TABLE I

THE EFFECTS OF LOW TEMPERATURES ON MATURE SHOOTS OF THREE SPECIES OF *Vaccinium* ALL LOTS EXPOSED FOR THREE HOURS IN A FREEZING CHAMBER

TEMPERATURE TREATMENT	*V. pennsylvanicum*	*V. canadense*	*V. corymbosum*
– 16° C.	Alive to tip buds All buds starting	Alive to tip buds Buds starting slowly	Alive to tips[1] Buds starting slowly Wood showing injury
– 20° C.	Alive to tip buds All buds starting	Buds not starting Wood color dull	Buds not starting Wood injury intensified
– 24.5° C.	Buds starting slowly Wood showing slight injury	Buds not starting Wood discolored	Buds not starting Wood discolored

[1] Except in the case of shoots killed at tips by freezing in the field.

It will be seen in this table that at – 16° C., *V. pennsylvanicum* was uninjured. In the other two species injury at this temperature was indicated by a slower pushing of buds. In *V. corymbosum* there was also a slight discoloration of the wood.

At – 20° C., *V. pennsylvanicum* still appeared uninjured with practically all buds starting to grow. *V. canadense* showed no buds starting and the normal greenish color of the wood was noticeably dulled. *V. corymbosum* was badly injured or killed with no buds starting and the wood had a water-soaked appearance.

At – 24.5° C., *V. pennsylvanicum* showed considerable injury with the buds starting slowly and the wood somewhat discolored. The other two species showed no sign of bud activity and the wood was darkened and had a water-soaked appearance. In *V. canadense* at this temperature the phloem was noticeably browned. Apparently this temperature was almost low enough to kill *V. pennsylvanicum* and did kill the other species.

As a further check on killing temperatures 15 to 20 shoots of *V. pennsylvanicum* and *V. canadense* were dug from beneath the snow at Cloquet on February 19, 1927, and frozen on February 28 for three hours at – 32° C. All of these shoots were killed except two shoots of *V. pennsylvanicum* which started a very few buds in a feeble manner after four weeks in the greenhouse.

These data show that the killing points of *V. corymbosum* and *V. canadense* at the end of October lie somewhere between – 16° and – 20° C., with *V. canadense* only slightly hardier than *V. corymbosum*. As *V. pennsylvanicum* was injured at – 24.5° in October and killed at – 32° in February,

it appears that the killing point of this species in October lies not far below −24.5° C. It is also evident that this species did not increase in hardiness to any appreciable extent after October.

In conclusion, it appears from the results of freezing tests that the killing points of all three species as grown in Minnesota lie fairly close together. This fact indicates that there is no great difference in the actual hardiness of the three species. Of still greater significance is the apparent inability of any of them to endure very low temperatures. Comparing the killing points of these *Vaccinium* species with those of orchard and forest trees which grow in northern climates (**1**, **2**, **5**) it is seen that the former develop relatively little cold resistance. Even the hardiest species, *V. pennsylvanicum*, apparently cannot endure exposure to such winter temperatures as commonly prevail in the regions to which it is native. As *V. pennsylvanicum* and *V. canadense* occur naturally over a more northerly and westerly range than *V. corymbosum*, in a region of low winter temperature but ordinarily with ample snow cover, it is safe to conclude that these two species are protected rather than inherently hardy. The adaptation of these species to cold climates appears to be largely an ecological one rather than any physiological adjustment of the tissues to extremely low temperatures. In the matter of avoiding cold injury in the fall, *V. pennsylvanicum* seems to have a slight advantage over the other two species, since it attains greater hardiness early in the fall when a few degrees of cold resistance may be sufficient to prevent killing back of the shoots. This fall hardiness, however, is probably only another result of the dwarf habit, the shorter terminal growths maturing earlier and becoming more hardened before severe freezing weather occurs.

With this relative lack of hardiness in all three species, it would seem futile to attempt to develop from this material tall hybrids which would be likely to grow late or to extend above the snow cover. If a truly cold resistant blueberry is to be developed apparently it will be necessary to search outside of these three species for hardy parent stocks.

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CERTAIN PHYSICOCHEMICAL PROPERTIES OF PINEAPPLE STEM COLLOIDS*

C. P. SIDERIS

(WITH EIGHT FIGURES)

The investigations reported in this paper were conducted with the purpose of obtaining certain information on the behavior of pineapple plant tissue colloids in health and disease. Some of this information is reported in another publication by the writer (**7**).

It was found (**6**) about two years ago that pineapple plants grown in water and soil cultures of different H-ion concentrations thrive best at pH values between 4.5 and 6.5. These findinge suggested that the inhibitory effect of H-ions above or below the values mentioned may have had some influence on the physicochemical properties of the pineapple tissue colloids. As such studies could not have been conducted very satisfactorily with the living tissues, it was thought best to obtain the fluid colloids and study them *in vitro*.

Methods of experimentation

Pineapple stems, from which the leaves, the extreme basal portion, and part of the exterior tissues of the base had been removed, were ground in a meat grinder. The pulpy mass was then placed in a fruit press and the fluids extracted by pressure. The extract was filtered through filter-paper for the removal of particles of plant tissue. The product thus obtained was centrifuged for further purification by means of a Sharples centrifuge, and then subjected to the treatments, as outlined later, for the separation of the different colloidal fractions. The reagents used for these treatments were in certain cases 0.1 normal NaOH and HCl, in others 0.1 normal NaOH and HNO_3, and 0.1 normal ammonium hydroxide and acetic acid. The pH of the resulting solution never went above 10.0 nor below 2.0.

The procedure followed for the treatment of the different colloid fractions may be illustrated by the following outline and diagram.

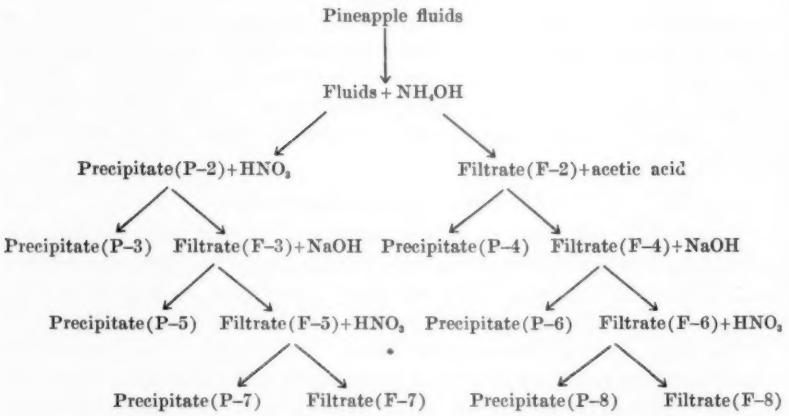
OUTLINE

1. Extracted pineapple fluids.
2. Treatment of pineapple fluids with NH_4OH. Formation of a precipitate (P–2) and filtrate (F–2).

* Technical paper no. 4 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

3. Treatment of precipitate (P–2) with HNO_3. Formation of a precipitate (P–3) and filtrate (F–3).
4. Treatment of filtrate (F–2) with acetic acid. Formation of precipitate (P–4) and filtrate (F–4).
5. Treatment of filtrate (F–3) with NaOH. Formation of precipitate (P–5) and filtrate (F–5).
6. Treatment of filtrate (F–4) with NaOH. Formation of a precipitate (P–6) and filtrate (F–6).
7. Treatment of filtrate (F–5) with HNO_3. Formation of a precipitate (P–7) and a filtrate (F–7).
8. Treatment of filtrate (F–6) with HNO_3. Formation of a precipitate (P–8) and filtrate (F–8).

DIAGRAM



Qualitative chemical tests of different fractions

The different fractions were analyzed qualitatively with the following results:

TABLE I

QUALITATIVE TESTS ON THE STEM COLLOIDS

TESTS	DIFFERENT COLLOID FRACTIONS					
	P–3	P–4	P–5	P–6	P–7	P–8
Biuret	+++	+++	—	—	+	+
Sulphur	—	—	—	—	+	+
Xanthoproteic	+	+	—	—	+	+
Molisch	—	—	+	+	—	—

The six different fractions may be placed in three groups as the result of their chemical behavior. P–3 and P–4 represent one and the same protein; P–7 and P–8 likewise, represent another protein, and P–5 and P–6 represent a carbohydrate.

Physicochemical properties of the different fractions

The different fractions were purified as thoroughly as possible by dialysis through parchment paper. Folded parchment paper cones containing the different colloidal fractions were immersed in four-liter beakers of distilled water. The water was changed three times a day. A piece of thymol, about the size of a pea, was kept in the water to prevent biological action. The electrical resistance of the dialysate was determined every

TABLE II

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-3 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION 24 HOURS		TURBIDITY	$AgNO_3$	$K_4Fe(CN)_6$	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH	AFTER TREATMENT	AFTER STERILIZATION				
	cc.	cc.	pH	pH				
1	5.0		2.25	2.30	–	–	–	Soluble
2	4.4		2.40	2.60	–	–	–	"
3	3.8		2.50	2.65	–	–	–	"
4	3.2		2.70	2.85	+	–	+++	Insoluble
5	2.6		2.80	3.20	++	–	++	"
6	2.0		3.15	4.10	+++	–	++	"
7	1.4		4.10	4.65	++++	–	+	"
8	0.8		5.00	4.85	++++	–	–	Insoluble (isoelectric point)
9	0.45		5.80	4.85	++++	–	–	Insoluble
10	0.22		6.20	4.85	++++	+	–	"
11	0	0	6.47	5.10	++++	+	–	"
12		0.3	6.65	5.50	+++	++	–	"
13		0.7	6.70	5.60	+++	++	–	"
14		1.3	6.90	6.05	++	+++	–	"
15		1.9	7.15	6.20	++	+++	–	"
16		2.5	7.30	6.60	+	+++	–	"
17		3.1	7.50	7.00	–	–	–	Soluble
18		3.7	7.80	7.50	–	–	–	"
19		4.3	8.10	7.80	–	–	–	"
20		4.9	8.60	8.00	–	–	–	"

time the solution was changed. After this value had risen from 200 to 15,000 ohms, the nitrate content from the added HNO_3 of the dialysate, was also determined. It was found that when an electrical resistance of about 20,000 ohms had been reached, the nitrate content of the dialysate was considerably less than one part per million. At this stage the different fractions were removed from the bags and studied for their physicochemical behavior.

Each fraction was made up to a certain volume with distilled water. This was acted upon, then, by a mechanical stirrer, in order to disperse evenly through the solution, the different sizes of the colloidal particles,

TABLE III

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-4 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION	TURBIDITY	$AgNO_3$	$K_4Fe(CN)_6$	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH					
	cc.	cc.	pH				
1	5.0		2.10	–	–	–	Soluble
2	4.5		2.15	–	–	–	"
3	4.0		2.20	–	–	–	"
4	3.5		2.25	–	–	–	"
5	3.0		2.35	–	–	–	"
6	2.5		2.40	–	–	–	"
7	2.0		2.45	–	–	–	"
8	1.5		2.80	–	–	–	"
9	1.0		3.20	+	–	+++	Insoluble
10	0.5		3.80	++	–	+++	"
11	0.25		4.40	+++	–	+++	"
12	0.125		4.60	+++	–	++	"
Check	0	0	4.65	++++	–	++	"
14		0.125	4.90	+++++	–	+	Insoluble (isoelectric point)
15		0.25	4.95	+++++	–	–	Insoluble
16		0.5	5.10	++++	–	–	"
17		1.0	5.30	+++	+	–	"
18		1.5	5.40	++	+	–	"
19		2.0	5.75	++	++	–	"
20		2.5	6.00	+	++	–	"
21		3.0	6.15	+	+++	–	"
22		3.5	6.35	+	+++	–	"
23		4.0	6.50	–	–	–	Soluble
24		4.5	6.65	–	–	–	"
25		5.0	6.75	–	–	–	"

before the solution was distributed into a number of Erlenmeyer flasks. Each flask received 100 cc. of the colloidal suspension drawn off by means of a pipette. The different flasks received, in addition to the colloidal suspension, a definite volume of 0.1 normal HNO_3 or NaOH. The number of cubic centimeters of the acid or alkali solution together with the pH value and turbidity that were produced are reported in tables II, III, IV, V and VI, and in fig. 7. In tables II and III and fig. 8, the reactivity of the different solutions with such salts as $K_4Fe(CN)_6$ and $AgNO_3$ also are recorded. The method adopted for determining the reactivity of these salts with protein P–3 and P–4 was as follows: Protein solutions, about 25 cc. of each, were taken into a dark room and there treated with 5 cc. of 1 per cent. concentration of either salt. The mixture was left to stand over night and was then filtered through hardened filter-paper. The residue was collected and placed in a solution having the same pH as that of the original solution. The intensity of the color of the precipitate served as an index of its reactivity. It may be added that before removing the precipitate from the filter-paper it is necessary to wash it with distilled water repeatedly until all traces of the unaffected salt have been removed. Equally good results were obtained when protein suspensions of different

TABLE IV

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-7 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION	TURBIDITY	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH			
	cc.	cc.	pH		
1	4.0		2.63	–	Soluble
2	3.5		2.67	–	"
3	3.0		2.70	–	"
4	2.5		2.88	–	"
5	2.0		3.00	+	Insoluble
6	1.5		3.13	+	"
7	1.0		3.33	++	"
8	0.5		4.16	+++	"
9	0.25		5.32	+++	"
10	0.125		6.45	++++	Insoluble (isoelectric point)
11	0	0	7.30	+++	"
12		0.125	8.65	++	"
13		0.25	9.45	+	"
14		0.5	9.90	–	Soluble
15		1.0	10.42	–	"
16		1.5	10.58	–	"
17		2.0	11.00	–	"

hydrogen-ion concentrations were treated with the above salts and left in darkness over night without subjecting the resulting solution to filtration. The intensity of the color that develops in the solutions after they have been exposed to the light may serve, in this case also, as an index of the reactivity of the salts and protein.

TABLE V

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-8 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		pH	TURBIDITY	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH			
	cc.	cc.			
1	5.0		2.00	–	Soluble
2	4.5		2.10	–	"
3	4.0		2.35	–	"
4	3.5		2.45	–	"
5	3.0		2.55	+	Insoluble
6	2.5		2.75	+	"
7	2.0		3.10	++	"
8	1.5		3.50	++	"
9	1.0		3.90	+++	"
10	0.5		5.35	+++	"
11	0.25		6.65	++++	Insoluble (isoelectric point)
12	0.125		7.20	+++	"
13	0.	0.	7.50	++	"
14		0.125	7.95	++	"
15		0.25	8.75	+	"
16		0.5	9.82	–	Soluble
17		1.0	10.37	–	"
18		1.5	10.85	–	"
19		2.0	11.00	–	"

Discussion of results

The results presented in tables II, III, IV, V and VI indicate that the difference in the physicochemical behavior of protein colloids and carbohydrate colloids is quite pronounced. The former react amphoterically and have a definite isoelectric point, whereas the latter do not. Different proteins may have different isoelectric points as is the case with fractions P-3 and P-4, on the one hand, and P-7 and P-8 on the other.

The amphoteric behavior of the two proteins is due, according to LOEB (3) and others, to their component reactive groups, namely, the amino (NH_2) and carboxyl (COOH) radicals, the former behaving as a cation, and the latter as an anion. In the case of the carbohydrate colloids, where

usually only one reactive group occurs, that is, the carboxyl radical, the behavior can not be amphoteric but points only in one direction. The experimental data are in complete agreement, therefore, with the theoretical expectations.

TABLE VI

BEHAVIOR OF SAMPLES P-5 AND P-6 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION	TURBIDITY
	0.1 N. HNO_3	0.1 N. NaOH		
	cc.	cc.	pH	
1	12.0	10.0	2.30	++++
2	8.0		2.50	+
3	6.0		2.90	+
4	4.0		5.30	++
5	2.0		6.70	++
6	1.0		7.2	+++
7	0.5		7.3	+++
8	0.0	0.0	7.4	++++
9		0.5	7.6	++++
10		1.0	7.7	++++
11		2.0	7.9	++++
12		4.0	8.4	++++
13		6.0	8.5	++++
14		8.0	8.6	++++
15		10.0	8.8	++++

During the different studies both the protein and carbohydrate colloids were subjected to various treatments. One hundred cc. of suspensions of protein P-3 and P-5 of pH values above and below the isoelectric point were treated with 10 cc. of 0.1 molal NaOH and HCl. The water of the suspension was evaporated at room temperature and the residue containing NaCl crystals and protein was examined under the microscope. The examination proved that at pH values above the isoelectric point of the protein the protein occupied part of the central portion of the crystal, whereas the arrangement was reversed at pH values below the isoelectric point, as shown by figs. 1, 2 and 3. At the isoelectric point the protein forms transparent thread-like structures, which, under the microscope, are transparent flakes without any definite arrangement.

When the carbohydrate colloids, of fractions P-5 and P-6, dissolved in a beaker or other container, were treated at intervals with drops of 0.1 normal NaOH, it was found that certain membranous spherical or ring bodies are formed, as shown in fig. 4. These bodies are hollow inside; that

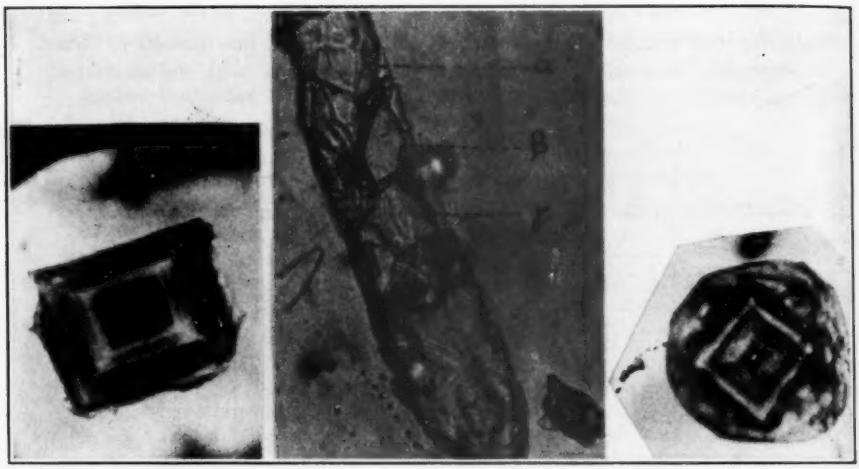


FIG. 1. Sodium chloride crystals in pineapple protein (P-7) suspension, at pH values above 6.5.

FIG. 2. Isoelectric pineapple protein (P-7) in an imperfect crystalline (α, β, and γ) form.

FIG. 3. Sodium chloride crystals in pineapple protein (P-7) suspension at pH values below 6.3.

is, the solid substance that enters into their composition does not fill all the space but like a rubber balloon forms a membranous shell. The thickness of the membrane of such bodies may vary from 0.5 to 0.05 mm. They may remain in the solution from a few seconds to many minutes depending on the acidity or alkalinity of the solution. If the solution is too acid their duration is short; if, however, it is but slightly acid or close to neutral they may last for many minutes and possibly hours. Their formation may be explained as follows: The COOH radical of this colloid fraction being chemically highly reactive is able to enter into a chemical combination with substances having an opposite electric charge and to form salts which may or may not be soluble. In this particular case the COOH reacting with NaOH according to the equation,—$RCOOH + NaOH \rightarrow RCOONa + H_2O$,—forms a certain insoluble salt. Whether the reaction, as stated, represents the actual conditions is not known. Qualitative tests of the carbohydrate colloid gave a positive test for calcium. The presence of calcium suggests that the substance may be a calcium pectate or some other similar salt. Such a salt when acted upon by an acid or a base may behave as follows:

(1) $(RCOO)_2Ca + 2HNO_3 \rightarrow 2RCOOH + Ca(NO_3)_2$.

(2) $2RCOOH + Ca(NO_3)_2 + 2NaOH \rightarrow (RCOO)_2Ca + 2NaNO_3 + 2H_2O$.

These reactions represent the conditions that might possibly develop with the different treatments. The equations are merely illustrative, and the balancing should not be interpreted as indicative of precise knowledge of the reactions.

The spherical or doughnut shaped bodies that are formed, when this carbohydrate colloid is treated with NaOH, may be the insoluble salt of calcium pectate or some other similar substance. The physicochemical conditions that enter into their formation may be explained as follows: The COOH radical upon coming in contact with the NaOH of the solution forms water while at the same time the H-ion is replaced by Na or Ca. With the replacement of the H-ion by Ca the membrane of the body or calcium pectate salt is formed. The water that is being formed is apparently sur-

FIG. 4. Carbohydrate colloid bodies of spherical and ring shapes formed by adding drops of 0.1 N. or 1.0 N. NaOH in an acid suspension of the colloids. The bodies are membranous, containing in their cavities water and some salts.

rounded by the membrane with the Na or Ca ions projecting into it. The inert part of the colloid substance or R occupying the exterior surface of the membrane projects into the exterior acid solution. With conditions like these it is possible to have the membrane lasting in the acid solution from a few minutes to a few hours. The decomposition or dissolution of the membrane may be brought about by the interplay of osmotic forces. The H-ions that are present in the outside solution penetrate the membrane either slowly or rapidly depending on their concentration. Their penetration into the interior of the spherical body may reverse the reaction, that is, the H-ion may replace the Ca or Na ions and reestablish the original condition as follows: $(COO)_2Ca + 2HNO_3 \rightarrow 2COOH + Ca(NO_3)_2$.

According to these observations, then, the structure of a carbohydrate colloid body, such as the one under discussion, may be imagined as a hollow sphere the shell of which is formed by aggregated micellae held in close proximity by electrostatic forces as represented by the sketch in fig. 5. Membranous bodies of this type have been observed to form in different sizes, varying between 10 and 0.5 mm.

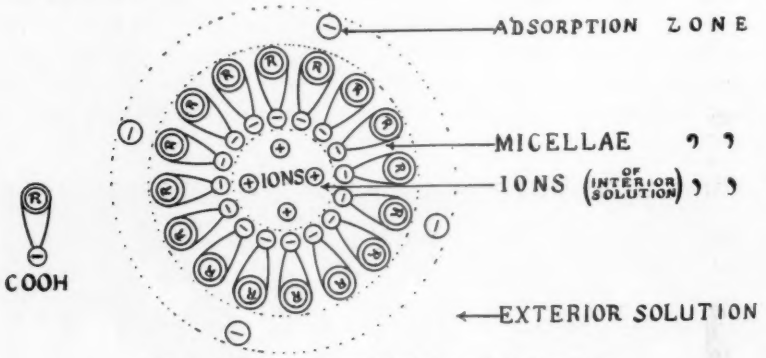


FIG. 5. Pattern of a carbohydrate colloidal particle.

The question now arises: Is it possible to have similar bodies formed with proteins at pH values above or below their isoelectric points? Assuming that isoelectric protein is like a collapsed rubber balloon or a flat membrane, is it not possible for such a membrane to assume a spherical or other similar form when suspended in solutions of a higher or lower pH from that of the isoelectric point of the protein? By analogy, one would expect in the case of the formation of spherical bodies, the COOH group to project inward at pH values above the isoelectric point and outward at pH values below this point. The position of the NH_2 group must be then, exactly the reverse of that of the COOH group, as in fig. 6. Although the work of previous investigators favors this view considerably, no one has ever been able to demonstrate that such a structure occurs in proteins. MEYER (**4**) has observed with starch and water the formation of a net structure made up distinctly of globules. HARDY (**2**) believes that concentrated gelatin jellies consist of drops of water suspended in a gelatin-rich phase. LOEB's (**3**) work on the swelling of proteins, suggests that proteins must undergo a certain modification in their isoelectric structure to make them hold water very tenaciously. From a theoretical consideration the formation of such membranous structures is possible only at pH values slightly above or below the isoelectric point of the particular protein. At considerably higher concentrations of H^+ or OH^- ions the electrostatic forces that keep the indi-

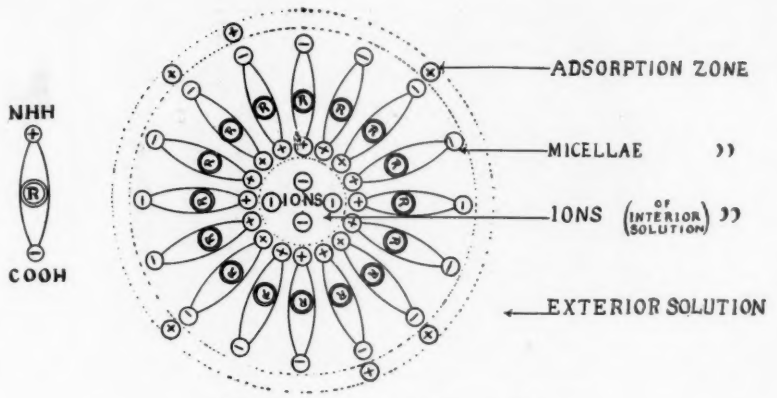


FIG. 6. Pattern of a protein particle.

vidual micellae together in a membrane-like structure are destroyed. The development of this condition is due to the increase of the electrical potential of the micellae. These, acted upon by high concentrations of H^+ or OH^-

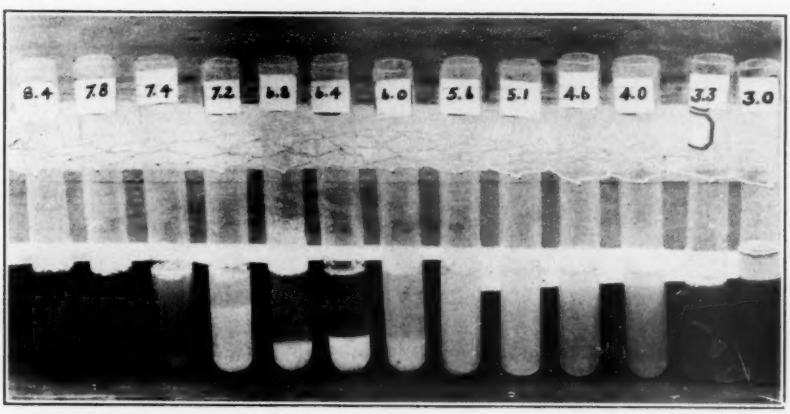


FIG. 7. Pineapple protein (P-7) in solution of different pH values. At 6.4, the isoelectric point, the protein precipitated completely; slightly at pH 6.8, 6.0, and 7.2; remained turbid at 5.6, 5.1, 4.6, 4.0, and 7.4, but dissolved completely at 7.8, 8.4, 3.3 and 3.0.

ions, become highly electrified, which condition makes them repel each other and disperse themselves thoroughly in the solution.

The phenomenon of dispersion and precipitation has been studied quite extensively by LOEB (**3**), NORTHROP (**5**), FAURÉ-FREMIET and NICHITA (**1**)

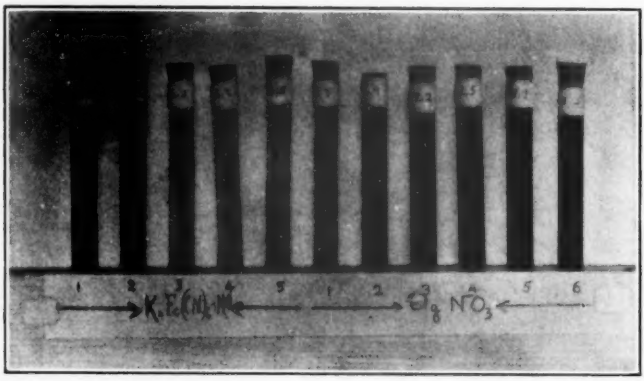


FIG. 8. Pineapple protein (P-7) treated with $K_4Fe(CN)_6$ and $AgNO_3$. It reacted well, and developed a yellowish green color at pH 5.6 and 6.2, which did not photograph well; at 3.1 it was green. With $AgNO_3$ it reacted well, as shown at pH 6.7–8.5.

and others. These investigators found that the stability of colloidal particles or bacteria in a suspension depends on the electrical potential of the solution. When this potential reaches a very low figure the particles or bacteria, as the case may be, become precipitated.

Further work on the structure of protein as well as carbohydrate colloids is necessary in order to obtain a clearer picture of their physical and chemical behavior.

Summary

The results obtained by the investigations reported in this paper indicate that it is possible to separate mixtures of colloids by making use of their isoelectric or critical precipitation point. Both carbohydrate and protein colloids may be separated in this way.

The writer expresses an opinion about the structure of colloidal particles which may be represented by a membranous body of spherical or other shape, the interior space of which contains water, with the reacting radical of the colloid and other ions in solution.

Pineapple stem protein has been obtained in an imperfectly crystalline form. The crystalline structure is flake-like, transparent, and macroscopically fibrous. The isoelectric point of this protein is at pH 6.4.

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THE QUANTITATIVE DETERMINATION OF CHLOROPHYLL[1]

F. M. SCHERTZ

(WITH TWO FIGURES)

Introduction

Up to the present time many methods have been used to determine the amount of chlorophyll present in a plant or in a given solution. Not one of the methods has been generally adopted by those who desire to estimate chlorophyll quantitatively. Each worker apparently has adopted a method of his own. It is clearly evident then that attempts at standardization should be made. The methods as given here are an attempt to place the estimation of chlorophyll on a sound basis, so that all workers may be able to compare their data. The results reported are not to be considered as final but only a report of progress in the development of better methods of quantitative research in this field.

Methods which have been used to estimate chlorophyll

No attempt will be made to refer to all of the methods which have been used for this kind of work but rather a brief description will be given of a few of the more important contributions.

Perhaps the first attempt at measuring the concentration of chlorophyll was made by MONTEVERDE (**7**) when he photographed the spectra of several different concentrations of the alcoholic extracts of green leaves. His purpose was not to discover a method for quantitative work but rather to ascertain the nature of chlorophyll spectroscopically.

In 1910 extinction coefficient values were used by MALARSKI and MARCHLEWSKI (**6**) to measure the concentration of colored solutions. Chlorophyll was converted into chlorophyllan (pheophytin) and the absorption of this solution was measured by means of the König-Martens spectrophotometer.

JACOBSON and MARCHLEWSKI (**3**) later used the photographic method in their study of the chlorophyllans from various leaves. By this method a direct comparison of the amount of chlorophyllan present could be made, for several photographs could be taken on the same plate. The method was used to show that chlorophyll consisted of two components, allo- and neochlorophyll. The procedure was then modified by JACOBSON (**2**) in order to determine the amount of the two chlorophyllans present in 1 gram or less of leaf.

[1] Soil-Fertility Investigations, U. S. Department of Agriculture, Washington, D. C.

Later MONTEVERDE and LUBIMENKO (**8**) used a spectrocolorimeter, which was specially constructed for their use by Leitz, in determining the amount of chlorophyll, carotin and xanthophyll present in the leaves of different plants.

WEIGERT (**10**) recognized the practical importance of knowledge regarding the spectrophotometric curves of the four chloroplast pigments. His figures show the extinction curves that are formed by solutions of the leaf pigments in pure acetone. He also explains how quantitative results may be obtained from such curves. No practical application, however, has ever been made of his results.

Considerable work has been done by HENRICI (**1**) on the chlorophyll content of alpine and lowland plants. Her methods were based upon those used by WILLSTÄTTER. The chlorophyll standard for the colorimetric determinations was an alcoholic solution of crude chlorophyll made from fresh nettle leaves. No attempt was made to place the amount of chlorophyll present in the leaves upon an absolute basis.

Total chlorophyll and total carotinoids were estimated in the red algae by WURMSER and DUCLAUX (**12**). They made no attempt to separate the two chlorophylls or to separate carotin from xanthophyll. The chlorophylls as such were determined spectrophotometrically by measuring the absorption at wave-length 670 mμ, and the carotinoids were determined by using wave-length 450 mμ. The amount of pigment present in the red varieties was expressed as 100 while the determinations for the green varieties were based upon the red and the results are given in per cent. The separation of the pigment was a modification of the procedure as given by WILLSTÄTTER and STOLL.

MAIWALD (**5**), by using methods based upon WILLSTÄTTER and STOLL's procedure, has determined the amount of chlorophyll present in potato leaves. A mixture of pure chlorophyll ($\alpha+\beta$) (obtained from WILLSTÄTTER) was used as a standard and comparisons were made by using a Dubosecq colorimeter.

By means of the spectrocolorimeter, LUBIMENKO (**4**) has quantitatively investigated the amount of chlorophyll present in marine algae. He used crystallized chlorophyll as a basis for his comparisons. The instrument is represented as being quite accurate, though no figures are given to show its degree of accuracy.

It will be observed that most of the methods used are only a modification of the procedure described by WILLSTÄTTER and STOLL (**11**). In their methods WILLSTÄTTER and STOLL separated chlorophyll into its two components, α and β, by converting them into their respective derivatives, phytochlorin *e* and phytorhodin *g*. The other workers have all determined only the total amount of chlorophyll which was present in the material.

Procedure

In this paper only the data for estimating total chlorophyll will be given, because the estimation of chlorophyll α and chlorophyll β by the methods of WILLSTÄTTER and STOLL have proved quite unsatisfactory. The manner of preparing a chlorophyll extract from fresh green leaves for use in such quantitative work has been presented (9). Pure chlorophyll ($\alpha+\beta$) has been used as a basis for the curves obtained by means of the Dubosecq colorimeter and also for those obtained by means of the Hilger wave-length spectrometer. In either case 0.0507[2] gm. of pure chlorophyll ($\alpha+\beta$) was dissolved in 50 cc. of ether. The chlorophyll in the ether was then saponified by shaking the solution for 15 min. or more with 10 cc. of cold concentrated methyl alcoholic potash. The ether was removed by evaporation, using reduced pressure. Distilled water was added to make a volume of 250 cc. Dilutions as required were made from this solution of potassium chlorophyllin ($\alpha+\beta$), and the pure chlorophyll was measured as potassium chlorophyllin.

The colorimetric method

The solution as described above was diluted and readings were made, using a combination of 3, 4, and 5 blue plus 10 and 20 yellow Lovibond slides as a standard. The chlorophyllin solution was matched against the Lovibond slides by comparing the depth of tint and not by attempting to match exactly the color of the slides, which would be impossible.

The combination of Lovibond slides used here does not exactly match the color of the chlorophyll solution. The writer used this combination to aid in determining the purity of his samples, for some fixed standard was absolutely necessary since no known pure chlorophyll was at hand. No combination of Lovibond slides will match all of the samples of chlorophyll, for the proportions of α and β vary. The tint of the chlorophyll solutions will vary accordingly. In quantitative work on fresh green leaves the color variations in the green pigments being tested will be even greater than in the case of pure chlorophyll. The ideal method, of course, is to use pure chlorophyll as a standard; but this will not be found to be practicable because pure chlorophyll is not available commercially, and moreover it is not advisable for each worker to prepare his own pure product. A company in America is now attempting to prepare pure chlorophyll and it is hoped that chlorophyll of known purity soon may be purchasable.

The results of colorimetric readings for six different samples are given in table I. In the case of samples 3, 4, 5, and 6, more than one weighing

[2] 0.0507 gm. was used, for when 0.0500 gm. of chlorophyll ($\alpha+\beta$) was dried at 100° C. it lost an average of 0.0007 gm.

TABLE I

COLORIMETRIC READINGS OF AQUEOUS SOLUTIONS OF POTASSIUM CHLOROPHYLLIN ($\alpha+\beta$) MEASURED AGAINST A COMBINATION OF LOVIBOND SLIDES

SAMPLE NO.	GRAMS PER LITER			
	0.20	0.15	0.10	0.05
	mm.	mm.	mm.	mm.
1	8.7	12.3	17.7	33.5[a]
2	8.5	11.6	16.1	35.0
3	9.1	10.6	17.4	33.5
3	8.5	.....	16.3	30.2
4	8.4	.....	16.8	32.1
4	9.0	12.2	17.8	31.9
5	7.5	.....	15.0	29.8
5	7.6	9.5	14.2	29.3
6	9.5	.....	17.8	32.8
6	7.7	9.6	15.7	28.6
6	9.6	12.8	19.6	34.9
Average	8.55	11.22	16.76	31.96

[a] Each number is an average of 3 readings on the colorimeter measured against the following combination of Lovibond slides, 3, 4 and 5 blue plus 10 and 20 yellow. Measurements were made on different days as well as different times of the day so as to get an average result.

was made and another set of readings was taken on a different day. This was done to obtain as good an average as possible, for it is almost impossible to duplicate exactly any set of readings on a given sample. Using the average of the figures for each concentration in table I, a curve has been

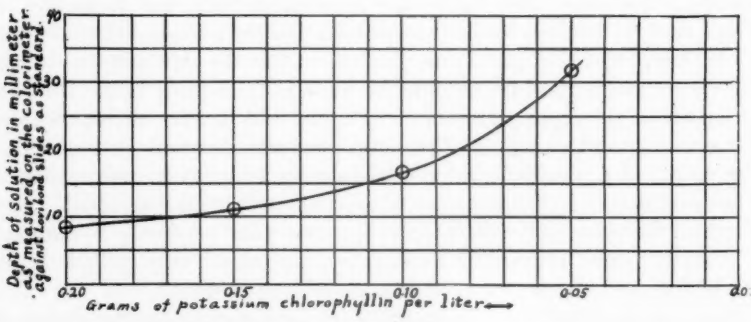

FIG. 1. Colorimetric determination of chlorophyll.

drawn, fig. 1, with grams of chlorophyll ($\alpha+\beta$) per liter represented on the x-axis and depth of solution in millimeters as measured on a colorimeter

represented on the y-axis. The curve may be used by those desiring to know approximately the amount of chlorophyll present in a given solution. However, it is advisable to use pure chlorophyll as a standard if it is obtainable.

In order to know the accuracy of the method the maximum error has been calculated for each of the four concentrations. The high, the low and the average reading together with their respective values in terms of chlorophyll are given in table II. Calculating, using the values for grams of

TABLE II

DATA, OBTAINED FROM TABLE I, USED IN CALCULATING THE PROBABLE ERROR OF A SINGLE DETERMINATION

	CONCENTRATION IN GRAMS PER LITER			
	0.20	0.15	0.10	0.05
	mm.	mm.	mm.	mm.
Highest reading	9.6	12.8	19.6	35.0
Lowest reading	7.5	9.5	14.2	28.6
Average reading	8.55	11.22	16.7	31.9

From the curve in fig. 1, these readings are interpreted in terms of grams of chlorophyll per liter respectively as follows:

0.173	0.133	0.083	0.047
0.235	0.175	0.120	0.054
0.200	0.150	0.100	0.050

chlorophyll per liter in table II, it is found that the maximum difference in the readings for concentration 0.20 is 0.235–0.173 = 0.062, and 0.062 = 31 per cent.; for 0.15 it is 28 per cent., for 0.10, 37 per cent., and for 0.05, 14 per cent.

From table I, the probable error of a single observation has been calculated. The calculations are rather lengthy and need not be given here, since the method of calculating is shown later in connection with the spectrometric method. For readings made with the concentrations 0.20, 0.15, 0.10 and 0.05, the respective probable errors in per cent. are $\pm$ 7.4, $\pm$ 7.3, $\pm$ 7.0 and $\pm$ 3.3.

The spectrometric method

The same solutions and concentrations used in the colorimetric method were used here also. The slit opening was set at 5, and a 200-watt electric light was used as the source of illumination. The widths of the absorption band (I) are given in table III. Only one set of readings was taken in

TABLE III

POSITION OF THE EDGES OF THE ABSORPTION BAND (I) OF PURE POTASSIUM CHLOROPHYLLIN ($\alpha+\beta$) (THICKNESS OF SOLUTION = 10 MM.)

SAMPLE	CONCENTRATION (GRAMS PER LITER)			
	0.20	0.15	0.10	0.05
1	668–605*	660–615	656–619	647–624
	667–607	659–615	654–619	646–624
	666–607	659–615	655–619	646–624
2	666–609	663–611	656–617	646–625
	666–609	662–612	656–617	647–623
	665–609	661–612	656–618	648–624
3	665–607	.....	656–614	650–621
	666–604	.....	656–616	649–621
	667–605	.....	655–616	650–622
4	665–607	.....	656–617	651–624
	664–608	.....	654–619	648–621
	663–608	.....	654–617	649–624
5	671–605	664–613	656–617	648–624
	670–605	662–612	656–618	646–624
	669–607	662–612	657–618	646–624
6	670–604	664–611	657–617	648–623
	670–604	662–611	657–617	647–623
	668–606	662–612	657–616	648–624
Average	667–606.4	661.7–612.6	655.7–617.3	647.7–623.3

* All readings are reported in mμ.

the case of each sample of chlorophyll ($\alpha+\beta$). From the average results in table III the graph in fig. 2 has been constructed. From this graph readings from a solution which contains an unknown amount of potassium chlorophyllin, may be interpreted in grams of chlorophyll per liter.

In order to find the maximum error in the spectrometric determinations in table III, the widths of the absorption band have been interpreted in grams of chlorophyll per liter and are recorded in table IV. From table IV the maximum difference in the determinations may be calculated as follows: For concentration 0.20 grams per liter it is 0.2340–0.182 = 0.0520 and $\frac{0.0520}{0.2055} = 25$ per cent.; for concentration 0.15 it is found to be 18.9 per cent., for concentration 0.10, 19.4 per cent., for 0.05, 53.2 per cent.

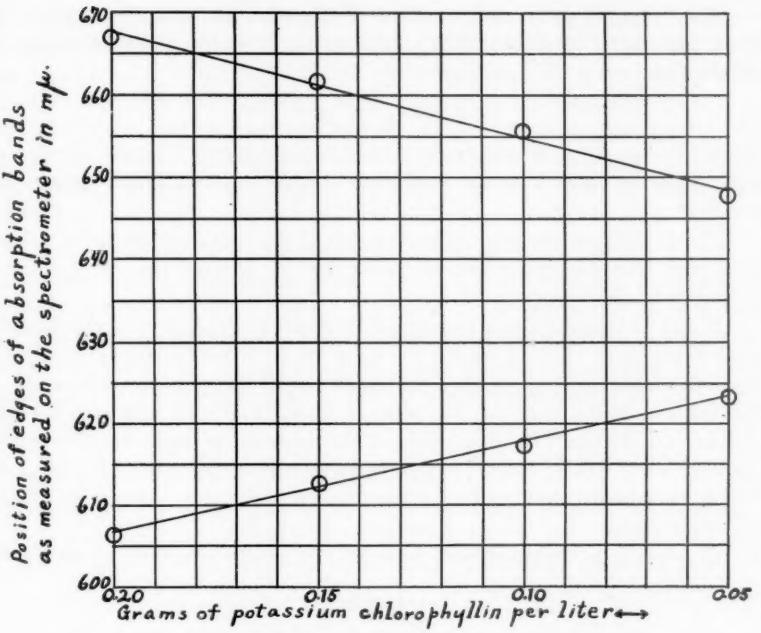

FIG. 2. Spectrometric determination of chlorophyll.

TABLE IV

DATA, OBTAINED FROM TABLE III, WHICH WAS USED IN CALCULATING THE ERROR OF A SINGLE DETERMINATION

SAMPLE	mμ (x)	mμ (x)	mμ (x)	mμ (x)
1	$60\frac{2}{3} = 0.200$	$44\frac{1}{3} = 0.133$	$36 = 0.098$	$22\frac{1}{3} = 0.040$
2	$56\frac{2}{3} = 0.183$	$51\frac{2}{3} = 0.159$	$36\frac{2}{3} = 0.107$	$23 = 0.042$
3	$60\frac{2}{3} = 0.200$	.....	$41 = 0.119$	$28\frac{1}{3} = 0.065$
4	$56\frac{1}{3} = 0.182$	.....	$37 = 0.102$	$26\frac{1}{3} = 0.057$
5	$64\frac{1}{3} = 0.233$	$50\frac{1}{3} = 0.158$	$38\frac{2}{3} = 0.107$	$22\frac{2}{3} = 0.041$
6	$64\frac{2}{3} = 0.234$	$51\frac{1}{3} = 0.162$	$40\frac{1}{3} = 0.116$	$24\frac{1}{3} = 0.048$
Lowest reading	0.1820	0.1330	0.0980	0.0400
Highest reading	0.2340	0.1620	0.1190	0.0650
Average reading	0.2055	0.1530	0.1081	0.0480

(x) = grams of chlorophyll per liter.
mμ = width of absorption band in mμ.

The probable error of a single determination has also been calculated from table III. The calculations will not be given but the results may be obtained by using the equation:

$$r = 0.6745 \sqrt{\frac{\Sigma\ V^2}{N-1}}$$

in which r is the probable error, 0.6745 is a constant, N is the number of determinations and V is the variation of each reading from the average reading.

The probable error for a concentration of 0.20 gm. per liter is ± 7.5 per cent., for 0.15 it is ± 5.8 per cent., for 0.10 it is ± 5.0 per cent., and for 0.05 it is ± 14.0 per cent.

It will perhaps be best to give an example to explain how the spectrometric graph is used. Using a given solution of potassium chlorophyllin the absorption band is found to extend from 620–652 mμ; then the width of the band is 32 mμ. In fig. 2 a place is found where the distance between the lines is 32 mμ. By inspection of the figure it is seen that this corresponds to 0.08 gm. of chlorophyll. So the solution, the absorption bands of which have been measured in the spectrometer, contains 0.08 gm. of chlorophyll per liter.

In table V a comparison is made of the results obtained by using the

TABLE V

COMPARISON OF THE ACCURACY OF THE COLORIMETER AND THE SPECTROMETER

ERRORS OF DETERMINATION	CONCENTRATION OF POTASSIUM CHLOROPHYLLIN IN GRAMS PER LITER			
	0.20	0.15	0.10	0.05
		Colorimeter results		
Maximum error	31 per cent.	28 per cent.	37 per cent.	14 per cent.
Probable error	± 7.4	± 7.3	± 7.0	± 3.3
		Spectrometer results		
Maximum error	24.0 per cent.	18.9 per cent.	19.4 per cent.	53.2 per cent.
Probable error	± 7.5	± 5.8	± 5.0	± 14.0

colorimeter with the results obtained on the spectrometer. It is seen that the results on the colorimeter are best when the concentration is about 0.05 gm. per liter while the results on the spectrometer are best with a concentration of 0.10 to 0.15 gm. per liter.

In the absence of better methods for the quantitative determination of chlorophyll either method should give results which are quite satisfactory. Methods have been used here in which it is not necessary to use pure chlorophyll as a standard because of the difficulty of obtaining this pigment pure.

The preparation of pure chlorophyll would be quite a task for many workers who are interested in knowing something regarding the pigment content of plants which are being investigated. Some of the difficulties in preparing pure chlorophyll will be taken up at a later time. The methods as outlined should at least aid in any preliminary investigation of chlorophyll. Of course, the ideal standard is pure chlorophyll; but until the pure pigment is obtainable commercially workers will have to be contented with other methods.

Stability of potassium chlorophyllin solutions

In connection with methods for estimating chlorophyll, investigators should know something regarding the stability of chlorophyllin solutions. Solutions of chlorophyll which had been saponified with methyl alcoholic potash were kept for ten days and the resulting decomposition is shown in table VI. The combination of Lovibond slides described under the colorimetric method was used here to obtain the colorimetric readings.

TABLE VI

KEEPING QUALITIES OF POTASSIUM CHLOROPHYLLIN, STORED AT ROOM TEMPERATURE IN DARKNESS, COLORIMETRIC METHOD

DATE	NUMBER OF DAYS	SAMPLE			
		1	2	3	4
		mm.	mm.	mm.	mm.
January 17	0	24.9	27.5	25.6	24.8
January 20	3	26.3	32.9	31.2	29.3
January 23	6	42.3	47.4	38.2	32.2
January 27	10	49.0	61.0	49.0	41.0

TABLE VII

KEEPING QUALITIES OF POTASSIUM CHLOROPHYLLIN STORED IN THE ICE BOX IN DARKNESS

DATE	NUMBER OF DAYS	READING IN MM.
June 10	0	10.3
June 12	2	10.3
June 26	16	11.0
July 22	42	11.4
August 14	65	11.7

However, a solution which had been kept in the ice box showed very little decomposition (table VII) even when stored for 65 days. The safer

practice would be to estimate the chlorophyll within a day or two after it had been prepared from the leaf material, meanwhile keeping the solutions stored in the ice box.

Potassium chlorophyllin is one of the most easily prepared of the chlorophyll products and is much more stable than any of the chlorophyll solutions, consequently it has been used as a basis for the determination of chlorophyll. Also, in separating chlorophyll from the yellow accompanying pigments it is necessary to saponify the chlorophyll to chlorophyllin. If any other derivative of chlorophyll were used in the estimation of chlorophyll our methods of separating the pigments would have to be modified considerably. Consequently, the methods as offered in this paper have been based upon the use of potassium chlorophyllin.

Discussion

This paper is concerned primarily with the methods now available for determining the amount of chlorophyll. It seems desirable in conclusion to say something about the future possibilities of determining chlorophyll. The methods as given here are not very accurate, being only good enough for preliminary work. Many problems concerning the estimation of chlorophyll demand an accuracy as great as has been obtained in determining carotin and xanthophyll. Data showing the complete spectrophotometric curves for carotin and xanthophyll have been obtained at the Bureau of Standards and it is hoped that this data will soon be published.

Before much more progress can be made with chlorophyll it will be necessary to have a complete spectrophotometric curve of chlorophyll α and chlorophyll β. This can be accomplished only after much more is known about chlorophyll than we know at the present time. Practically nothing is known at present about how to prepare a solution of chlorophyll so that its spectral transmission properties can be measured before the chlorophyll is altered by the solvent or by the light used in the study.

Before satisfactory solutions of chlorophyll α or β can be prepared, pigments of undoubted purity must be obtained. The preparation of such pure pigments demands most painstaking chemical technique. Thus far only one chemist has succeeded in preparing solutions of the permitted food dyes pure enough for satisfactory spectrophotometric analysis. It is not to be expected, then, that the preparation of pure chlorophyll α and β will be easily accomplished. Satisfactory spectrophotometric curves for the chlorophyll pigments will be obtained only with great effort and at considerable cost.

Difficult as the problem is, it can be solved if only the determination to solve it is present, but the solution will not be an easy one. While such

work has its practical value, the first consideration should be to gain more information about a substance so prominent everywhere in nature. Methods for the absolutely accurate quantitative determination of the green plant pigments will make it possible to know much more about the rôle of chlorophyll in everyday life. It is hoped that many plant physiologists will become interested in the nature and properties of this pigment which is known to play so important a rôle in plant life. It may be possible that a study of the effect of different wave-lengths of light upon the molecule of chlorophyll in pure solution will reveal much concerning the real nature of light effects upon plant growth and the nature of chlorophyll itself. The work will be most difficult, but since methods are being developed for investigations of this kind, sustained efforts should be made toward a detailed knowledge of the rôle of chlorophyll. The possibility for new discoveries is great.

This paper is only a report of progress in the efforts made to estimate chlorophyll with accuracy, efforts which have not yet met with entire success. More means should be available for purely scientific studies of the nature and functions of the α and β chlorophylls. At present we will have to be contented to work with the tools we have until more and better investigators become seriously interested in chlorophyll problems. Before such serious interest may be developed in the field of chlorophyll chemistry, it may be necessary to develop new points of view of the possible functions of chlorophyll, and the broad significance it may have in the whole realm of plant and animal life.

Summary

1. A brief description is given of the more important methods which have been used to determine chlorophyll quantitatively.

2. A method for determining chlorophyll colorimetrically is described and a graph is given from which quantitative data may be computed.

3. A spectrometric method of determining chlorophyll is described and a graph has been constructed from which chlorophyll may be quantitatively determined.

4. The colorimetric method is more accurate at concentrations of about 0.05 gm. per liter while the spectrometric method is more accurate at concentrations of 0.10 to 0.15 gm. per liter.

5. Chlorophyll solutions which have been saponified with methyl alcoholic potash should not be allowed to stand for any length of time before their pigment content is estimated, since the saponified chlorophyll is rather unstable.

6. The spectrophotometric method offers great promise of being a very accurate method for determining chlorophyll, though the data for the method are yet to be obtained.

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BIOCHEMICAL STUDIES ON SEED VIABILITY
I. MEASUREMENTS OF CONDUCTANCE AND REDUCTION

R. P. Hibbard and E. V. Miller

(with two figures)

Introduction

Previous work (**9**) on this problem led to the conclusion that a correlation existed between electrical conductivity of seed extracts and seed viability. With the broadening out of the work and an improvement in the method it was hoped that this conclusion would be still further substantiated. The studies here reported therefore deal with further investigations on this and related lines.

Such work, based on permeability, must necessarily be far from complete in its elucidation, since we have as yet no precise knowledge of the phenomenon itself. That permeability exists in seeds is self-evident but no one has yet offered a plausible explanation. It has been known for some time that a definite relation exists between the permeability of the cell membrane and its injury (**19**), and it might readily be inferred that a variation in degree of permeability would be correlated with similar variations in degree of injury. The degree of permeability may be indicated by measuring the resistance of the solution in which the seeds have been immersed. Many tests have shown that dead seeds are more permeable than viable ones, so that one might naturally expect that a variation in permeability would be correlated with variations in germination. Thus, a series showing variations in germination in increments of two or three per cent., on the range of zero to one hundred, might be worked out. Obviously, this would be more desirable than merely to know that a seed is dead or is viable.

Historical

Conductance methods

The use of conductance methods for measurements in physiological research dates back to the work of Edward Weber (**26**) in 1836 and Du Bois-Reymond (**8**) in 1849. Ranke (**21**) in 1865 noticed the decrease in resistance in plant and animal tissues upon death.

Brooks (**2**) in 1923 worked with *Laminaria*, yeast, bacteria, and blood cells. He showed that during the progress of heating of *Laminaria* the "net conductance" approached a constant value which he considered indicative of death. With *Bacillus coli*, his results are rather variable.

JOHNSON and GREEN (**12**) have shown that upon death the conductivity of yeast cells increases, this being due both to exosmosis of salts and to decrease in size of cells.

The outstanding investigator in the field of electrical conductivity measurements of permeability in plant cells is OSTERHOUT (**18**), who recommends this method and shows that the results do not vary more than one per cent. from the mean. In a later work (**19**) he pointed out that the time curve expressing the increase in permeability of *Nitella* during the progress of dying is practically the same whether derived from measurements of exomosis or electrical resistance.

OTHER METHODS

The problem of viability of seeds has been attacked from several different points of vantage. DARSIE and ELLIOTT (**7**), working with PEIRCE in 1914 noticed that the heat of respiration was greater for live seeds than for dead ones. Heat measurements were made under adiabatic conditions, the seeds being placed in silvered Dewar flasks under suitable conditions for germination. These authors claimed that there was a "normal temperature" for each species of plant and that departure from this temperature indicated departures from the best conditions of the organism. Excess of normal temperature indicates infection, while subnormal temperature is indicative of lessened vigor, usually due to increased age. The authors did not make any great claims as to the accuracy of this method for determining viability, but it is evident that seeds of high, low, and medium viability only could be thus determined.

LESAGE (**14**) evolved a method in which seeds were soaked in solutions of KOH of strengths varying from normal to N/682. Non-germinating seeds imparted a color to all solutions, while the viable seeds colored the strong solutions and those down to N/32, but had no noticeable effect upon weaker solutions.

Many workers have attacked the problem of seed viability from the standpoint of enzyme activity. KASTLE (**13**) in his classical work on oxidases, states that peroxidases and catalases are even more widely distributed in living tissues than are oxidases.

Peroxidases attack hydrogen peroxide and liberate atomic oxygen. Their presence may be demonstrated by the fact that they cause bluing of guaiacum. Samples are ground, a drop or two of guaiacum added, and two to three cubic centimeters of neutral hydrogen peroxide introduced. If the peroxidases are present, the guaiacum becomes blue, the intensity of color being proportional to the degree of enzyme action.

McHargue (**16**) has applied this peroxidase test to seeds of corn, hemp, tomato, oat, cow pea, soy bean, castor bean, and lettuce. He claims that these seeds, when exhibiting zero germination, displayed no peroxidase reaction. He goes still further and claims that the peroxidase reaction might be used for seed testing and that seeds of high, low, and medium viability might be thus classified.

On the other hand, Brocq-Rosseu and Gain (**1**) studied the peroxidase activity of seeds ranging from two years to five thousand years old. They found peroxidase activity exhibited in a wheat sample 2000 years old and claim that wheat will retain peroxidase activity 100 years after it loses its ability to germinate.

The enzyme catalase has also been employed for determining viability of seeds. This enzyme attacks hydrogen peroxide and liberates molecular oxygen. A known weight of the powdered sample is mixed with a known volume of neutral hydrogen peroxide and the volume of oxygen evolved is measured. Crocker and Harrington (**6**) determined the catalase activity of Johnson grass and Sudan grass seeds, of the same sample, before and after they had germinated. It was found that the catalase activity increased with germination, thus paralleling respiratory intensity. With *Amaranthus* seeds no correlation was found to exist between catalase and respiratory intensity, vitality, and age. They conclude that generally there is a close correlation between catalase activity and respiratory intensity, but not a very close correlation between either of these and the vitality of the seed or the vigor of the seedlings.

Němec and Duchon (**17**) demonstrated a close correlation between catalase activity and viability of seeds, being able to obtain a difference between seeds varying not more than two or three per cent. in germination. These workers employed cereal grains, legumes, and other seeds.

In 1924 Marotta and Kaminka (**15**) found that Němec's catalase method could not be applied to wheat seeds. Shull and Davis (**24**) have found a relationship between catalase activity and delayed germination in *Xanthium* seeds. There is a decrease in catalase activity in delayed germination. In an earlier work with *Xanthium* seeds, Shull (**23**) showed that oxygen accelerates germination and that a greater amount of oxygen is absorbed with the seed coats removed. Crocker (**5**) showed that oxygen increases respiration and in this way initiates germination.

Since catalase is an oxidizing enzyme and believed to participate in some manner in respiration, it may be this connection with vital activities that suggested the large amount of work attempting to correlate catalase with viability.

All of the evidence herein quoted seems to furnish direct confirmation of the conception of enzymatic activity and its relation to vital phenomena

as held by PALLADIN (**20**, p. 173) who says: "Life processes are not to be interpreted simply as enzymatic activity." This writer continues to show how organisms might be killed without destroying the enzymes and that enzyme activity is always exhibited by freshly killed tissues where precaution was not previously taken to destroy the enzymes, and that the only difference between enzyme activity in living tissues and dead ones is that in the former they are organized in their work.

We have thus attempted to review briefly the work that has been done toward shortening the method of determining viability of seeds. The majority of the workers quoted here have sought to correlate enzymatic activity with viability. Though enzymes are originally associated with living organisms, we have attempted to show in this historical review that they are not necessarily to be taken as indices of life in organisms.

Catalase and peroxidases might be classified as respiratory enzymes and for this reason may be thought to be concerned with vital phenomena. On the other hand, both dead and living seeds respire and respiratory measurements would be of little value for determining viability. Again any carbon compound might be oxidized to carbon dioxide and this process thus be taken for one of respiration.

The deeper we penetrate into this problem the more complex it grows. It practically resolves itself into the question of "When is a seed dead?" or it becomes a matter of evolving a physico-chemical means of determining the difference between life and death. Has this been determined even for the higher organisms? We are told that death is gradual even in man.

Whether there be a chemico-physical difference between life and death, it seems that there should be a correlation between viability and permeability of plant cells. It seems reasonable to suppose that non-living cells should be more permeable than living ones and that the salts should leach out more rapidly from the dead cells than from the live ones. The amount of salts diffusing out of the cells of the seeds should then modify the resistance of distilled water in which the seeds were soaking and this change of resistance could thus be measured electrically. An attempt was made to measure this change in resistance and to correlate it with viability of seeds. Suitable apparatus was assembled for making the determination.

Apparatus and method

With the exception of a few minor changes the apparatus recommended in the paper by HIBBARD and CHAPMAN (**10**) was used. The immersion type of electrolytic cell, the best for this kind of work, was thrust into the liquid in which the seeds were soaking.

A stirring apparatus was set up so that possible error due to hand agitation would be avoided. A weighed amount of seeds was poured into

each of the four clean pyrex beakers containing 100 cc. of liquid. The beakers were then placed in a constant temperature water bath at 25° C. A friction-drive universal motor was connected to the four stirrers so that the four samples should be run simultaneously. The electrodes which were immersed in the beakers were connected to a four-way switch for greater convenience.

The most radical departure from the method of FICK and HIBBARD (**9**) was made in the substitution of dilute $KMnO_4$ solutions for the conductivity water in the measurements of resistance. A number of references have been made above to the work on enzymatic activity as related to viability. KASTLE (**13**) mentions the fact that catalases, which occur in plant extracts and which liberate molecular oxygen from hydrogen peroxide, will reduce other oxidizing agents, among them being $KMnO_4$. While making some preliminary tests for catalase it was noticed that seeds reduced very dilute solutions of $KMnO_4$ at different rates.

The thought occurred that along with electrolytes there were substances of organic nature leaching out of the seed and hence not measurable by conductivity methods as previously used. This being true, the distilled water might be replaced by the $KMnO_4$ solution and the change in resistance noted as the permanganate is reduced.

Electrical resistance of solutions containing seeds of varying viability

EXPERIMENT I

Beakers containing 100 cc. of M/20,000 $KMnO_4$ were placed in the water bath, the electrodes inserted and connected, and the stirrers started. The seed samples were then added to each beaker, permitting one minute to elapse between the addition of subsequent samples, in order that the resistance readings might be made in the same order. The quantity of seeds used was one gram of timothy, or 50 peas, or 100 wheat seeds. Resistances were determined and the readings recorded at regular intervals. The results are presented in tables I, II and III. For the sake of brevity the figures which represent the resistance of the original $KMnO_4$ solution plus that of the salts and organic substances leached out of the seeds will hereafter be designated as "solution resistance."

The wheat seeds were obtained from the Farm Crops Department of Michigan State College. The variety was Red Rock and the samples varied in age. One hundred seeds were taken from each sample, after all broken, discolored, and infected seeds had been discarded. These were germinated and the percentage germination found was taken as that of the sample. In the above table four different samples having three different germination percentages were taken for the "solution resistance" tests.

TABLE I

SOLUTION RESISTANCE OF WHEAT IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
108	86	7361	7361
101	86	7364	7364
100	81	7241	7292
100	81	7361	
102	22	4663	5119
102	22	5576	

These garden pea samples were obtained from the Michigan Farm Bureau. The variety was Alaska. Those selected for this experiment varied in germination percentages, as may be seen from the table, from 97 to 63 representing samples of varying ages. History of these samples are not known, but the variety was good, and germination was especially high for the most recently harvested ones. The chief idea here as in the

TABLE II

SOLUTION RESISTANCE OF PEAS IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
110	97	12880	12985
110	97	13090	
106	93	16250	15945
106	93	15640	
107	93	16320	16145
107	93	15970	
111	92	16320	13355
111	92	16390	
112	90	11600	10800
112	90	10000	
109	83	9048	9729
109	83	10410	
103	76	10660	10535
103	76	8051	
105	75	11830	10072
105	75	8315	
104	63	5699	5518
104	63	5337	

other seed samples where the samples were not described in detail was to get good samples of different ages and different germination percentages.

TABLE III

SOLUTION RESISTANCE OF TIMOTHY IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
58	81	6340	6340
68	70	4970	4795
68	70	4620	
57	54	5974	5715
57	54	5456	
65	0	4205	4266
65	0	4327	

Samples 58 and 57 were obtained from Wisconsin. The year these samples were harvested is not known. Sample 65 came from Iowa, harvested in 1912. Sample 68 also came from Iowa, harvested in 1915 from the Agronomy plots.

Greater encouragement was received from the resistance measurements in $KMnO_4$ than from any in conductivity water. In table I the wheat shows true correlation. The viability varies directly with the resistance. In the case of the peas (table II) the samples of from 90 to 97 per cent. germination show high resistance. With but one or two exceptions the rest of the figures for resistance fall in their proper places in the table. The experiment with timothy (table II) is not entirely in accord with the first results.

EXPERIMENT II

Corn seeds from 8 different samples were next divided into two lots. Lot 1 was placed on a window ledge overnight where the temperature reached as low as 0° F. (Subsequent tests showed that these seeds did not freeze. Hence they will be used as checks against the killed seeds).

Lot 2 was kept in a hot air oven long enough to kill the embryo. The solution resistance of these two lots appear in table IV.

In column 2 of this table, opposite the sample numbers indicated in column 1, one may find the germination percentages for the seeds in the different samples. The solution resistance before and after the heat treatment is shown in columns 3 and 4. Fig. 1 represents the results of column 3 in graphical form.

TABLE IV

SOLUTION RESISTANCE OF LIVING AND DEAD CORN SEEDS

SAMPLE	GERMINATION	SOLUTION RESISTANCE (50 MIN.)	
		Lot 1 (alive)	Lot 2 (dead)
	per cent.	ohms	ohms
155	100	19670	17395
156	99	27410	24680
157	98	17250	14760
152	96	16810	15000*
150	94	14650	12385**
148	70	12170	12980
144	65	14920	10556**
154	1	6449	5712

* Duplicate discarded. ** Browned by heat.

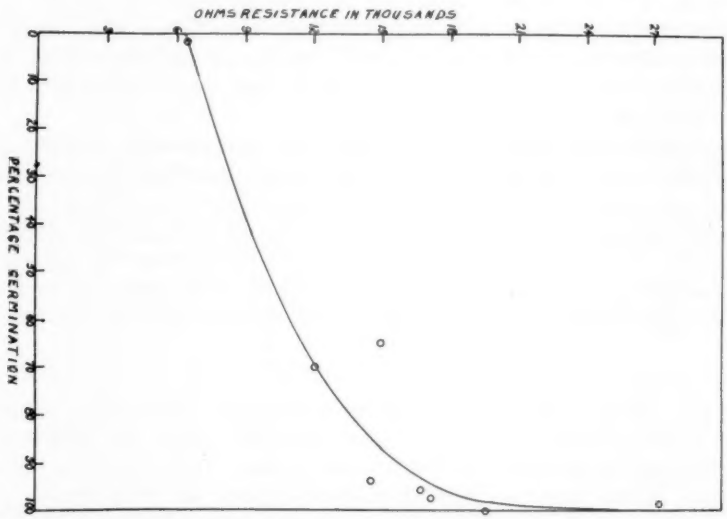

FIG. 1. Graph of relation of solution resistance to viability, from lot 1, table IV.

The samples used here were obtained from the Farm Crops Department of Michigan State College. The variety was Duncan. Eight groups were taken for the test.

Reduction of potassium permanganate by viable and non-viable seeds

In determining the solution resistance of seeds in potassium permanganate it was noticed that the permanganate was reduced at different rates

by different samples, the end-point being an easily recognized amber color. Resistance measurements taken at regular intervals did not take into account the color changes, being concerned with the changes in solution resistance only.

It was then decided to determine the time-rate of reduction of $KMnO_4$ by the seeds. One hundred corn seeds were ground to the fineness of meal, one-gram samples weighed out and permitted to stand in twenty cubic centimeters of distilled water for an hour. At the end of this time the mixture was filtered, one cubic centimeter of the filtrate drawn off and added to 0.5 cc. of M/800 $KMnO_4$. The time of adding the filtrate to the permanganate was recorded as well as the time when it was completely reduced.

The seeds were ground because the substances reducing the permanganate were dissolved in the water more readily and the process thus hastened. The cells would hardly be destroyed by this coarse grinding since it was performed in a meat chopper. Material must be ground with quartz sand in order to be reasonably sure of rupturing the individual cells. Grinding did not influence the relative rates of reduction by the different samples as was shown in several preliminary tests.

The relation of germination percentages to time-rate of reduction will be found in table V and fig. 2.

TABLE V

RELATION OF GERMINATION PERCENTAGES OF CORN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS (POWDER)

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
122	98.3	38.3
124	97.0	30.8
129	96.0	40.2
128	87.5	36.7
127	87.0	29.8
126	80.4	26.8
123	34.0	13.3
121	33.0	13.0
125	3.0	5.9

It was found that, with the exception of but a few of the high-germination samples, there was direct correlation between viability and time of reduction of permanganate. The higher the germination the longer the time required for complete reduction of the permanganate.

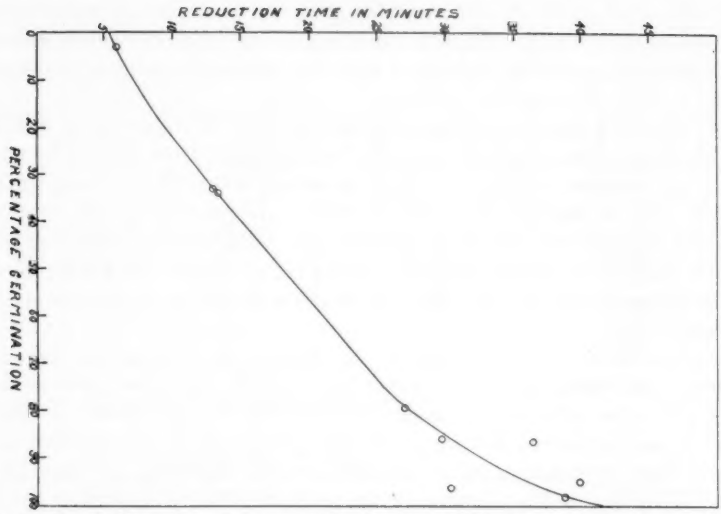

FIG. 2. Graph of relation of time-rate of reduction of $KMnO_4$ to viability, from table V.

A large number of samples was secured and the corn meal was passed through a twenty-mesh sieve before soaking. As was true in other experiments where a greater number of samples was employed, the results are not nearly so uniform. These results are presented in table VI.

TABLE VI

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF MEAL (20 MESH)

SAMPLE	GERMINATION	TIME TO REDUCE	SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.		per cent.	min.
139	100	21.9	150	94	15.9
142	100	18.5	153	93	15.5
156	99	16.5	160	93	14.1
146	99	10.9	143	83	17.9
147	99	14.3	159	79	23.5
140	99	14.2	148	70	16.5
157	98	12.5	141	71	10.3
152	96	22.5	138	65	18.3
158	96	17.9	144	65	10.9
151	95	21.9	154	1	19.0
145	94	12.0	149	0	7.5

For the purpose of shortening the method, grinding was dispensed with in another experiment and the seeds themselves were soaked in the permanganate. Ten seeds of corn were placed in twenty cubic centimeters of N/1000 potassium permanganate and the time recorded. The time was again recorded when the permanganate was completely reduced. It was found that if a few drops of N/10 oxalic acid were added to the mixture the end point would be closer and colorless instead of amber colored. The results may be found in table VII.

TABLE VII

RELATION OF VIABILITY OF CORN TO TIME-RATE OF REDUCING POTASSIUM PERMANGANATE (WHOLE SEEDS)

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
146	99	10.9
152	96	22.5
151	95	21.9
143	83	17.9
141	71	10.0
149	0	7.5

In the six samples included in table VII there is only one (146) which does not occur in the regular order. There is here almost complete correlation between viability and the reduction of potassium permanganate.

Another modification in the method was made. To hasten the time of reaction the seeds were first soaked for 12 hours in water, and 1 cc. aliquots withdrawn for the test instead of using the seeds. One cubic centimeter of this extract was treated with one drop of N/2 $KMnO_4$. The time required for complete reduction was recorded. The results will be found in table VIII.

TABLE VIII

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF WHOLE SEEDS

SAMPLE	GERMINATION	TIME TO REDUCE	SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.		per cent.	min.
155	100	23.0	167	81	20.5
161	99	23.0	168	65	19.0
162	99	21.5	171	25	12.0
163	98	24.5	173	2	7.0
172	97	11.5	169	0	18.0
164	86	24.5	170	0	12.0
166	83	21.0			

Here, too, low viability seemed to be consistent with the rapidity of reduction of potassium permanganate, although there is a great amount of non-uniformity in the table. The results in table IX were obtained in the same manner as those in table VIII. Here again the low-germinating seeds were first to reduce the potassium permanganate.

TABLE IX

RELATION OF VIABILITY OF CORN TO REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS OF WHOLE SEEDS

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
162	99	39
163	98	34
164	86	38
166	83	37.5
167	81	29.5
168	65	31
170	0	22
149	0	12
154	1	19
169	0	22

At the conclusion of this experiment on reduction of $KMnO_4$ by corn seeds some additional experiments were conducted with beans. The results (table X) exhibit a moderate amount of correlation. The figures for germination were obtained by selecting fifty normal-appearing seeds, sterilizing them, and testing for germination. This was done because it was learned that these were mixed samples, having been more or less adulterated by the growers. This is quite evident from the results of several different germina-

TABLE X

RELATION OF VIABILITY OF BEAN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
174	100	31.0
175	100	31.0
176	98	25.5
177	98	26.5
178	100	19.0
179	46	38.0
180	0	14.0

tions. In one set a hundred seeds were selected at random; in another the twenty seeds that had been used in the reduction of the $KMnO_4$ were subsequently germinated. The variation observed in table XI may also be due to the difference in methods applied.

In regard to the bean seed, these were of the Navy variety and were harvested in 1925. The varying germination percentages in these samples were due to selection. The samples showing highest germination were gathered, picked, and selected after harvesting and the rest were left on the ground for several days, exposed to the climatic conditions, being sampled on different days. In this way samples of varying germination percentages were obtained from seeds that had died or were infected to seeds of high or perfect germination.

TABLE XI

GERMINATION TEST OF BEANS

SAMPLE	GERMINATION		
	100 SEEDS	200 SEEDS SELECTED	50 SEEDS, SELECTED AND STERILIZED
	per cent.	per cent.	per cent.
174	90	100	100
175	89	95	100
176	85	85	98
177	76	90	98
178	27	65	100
179	15	15	46
180	0	0	0

As will be shown in the latter part of this paper, the reducing substance has been found to be present in large quantities in the seed coat of the bean. Accordingly, ten bean seeds selected from the seven different samples were soaked over night in distilled water, and the seed coats then removed and placed in 10 cubic centimeters of N/1000 $KMnO_4$, the time for reduction being recorded. The ten seeds, after the coats had been removed, were germinated. The germination percentages and the time-rate of reduction are to be found in table XII. Not only was direct correlation between germination percentages and rate of reduction found, but sample 179, which was out of the expected order as far as time relations were concerned in table X, appeared in its proper place in table XII. This experiment was repeated at a different time with similar results.

An experiment was next performed in which the three parts of the seed (seed coat, cotyledons, plumule and hypocotyl) were treated with the potassium permanganate solution. In this case there were employed 5 seed

TABLE XII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY THEIR SEED COATS

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
174	100	26
175	100	24
176	100	23
177	100	23
178	90	20
179	30	16
180	0	1

coats, 10 cotyledons, and 10 embryos. The figures for germination are those obtained from the 50 selected and sterilized seeds. The results in table XIII show almost complete correlation in all cases. The seeds germinating above 90 per cent. do not always occur in the proper order; yet they are usually higher in the table than those of low germination.

TABLE XIII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCING $KMnO_4$ BY DIFFERENT PARTS OF SEED

SAMPLE	GERMINATION	TIME REQUIRED FOR REDUCING		
		COATS	COTYLEDONS	EMBRYOS
	per cent.	min.	min.	min.
174	100	34.0	11.0	12.0
175	100	30.5	6.0	11.0
176	98	27.5	8.5	9.0
177	98	32.5	6.5	8.5
178	100	30.0	7.0	10.0
179	46	26.5	5.0	7.0
180	0	1.5	2.0	3.0

Studies of the nature of the substance

It is of interest to know the nature of this substance which reduces the potassium permanganate. REED (**22**) believes that the peroxidases in plant juices, having the power to absorb oxygen from oxygenases, will attack $KMnO_4$ in the same manner. BUNZEL and HASSELBRING (**3**) note that $KMnO_4$ may be reduced to a straw color by peroxides of manganese, and further to a clear solution by organic substances, but they hold that the oxidations are brought about by peroxides of manganese rather than by activated plant peroxidases.

That the reduction mentioned in this paper is not enzymatic may be demonstrated by first boiling the aqueous extract of seeds for fifteen minutes. The reduction will take place just as readily after boiling.

There is evidence supporting the view that these reducing substances may belong to the group of peptides, acid amides, or amino acids. If the proteins are precipitated by lead acetate and the excess lead removed by means of sodium carbonate, the filtrate will still reduce the potassium permanganate. Furthermore, an aqueous extract of the precipitate brought down by the lead acetate will not produce the reduction unless previously boiled with 10 per cent. HCl solution or incubated with pepsin solution.

Proteoses would remain in the solution after the proteins had been removed with lead acetate. However, phosphotungstic acid brought down no precipitate and it was assumed that in this case there were no proteoses present.

For a long time it was believed that ungerminated seeds contained no protein cleavage products. This view has now been changed by JODIDI (**11**) and co-workers who have found amino acids and peptides to be present in ungerminated kernels of maize, rye, wheat, and oats. BUSHEY (**4**) showed that frosted and "hailed" corn, besides being high in certain proteins, was also much higher in amide content than normal grains.

Discussion

A very detailed and extensive study of the conductance method as briefly outlined in the paper by FICK and HIBBARD (**9**) revealed that as a test for distinguishing between seeds of high, low, and medium germination it was very good. As a test for distinguishing difference in seeds varying in increments of 2 or 3 per cent. it is worthless and so far no method has yet been devised to obtain this degree of accuracy. There are some facts militating against the use of this method. It is quite expensive to begin with, and assumes that the operator has at least a technical and a more or less complete knowledge of the details of a set up for making conductance measurements.

Furthermore, the method presupposes that the electrolytic content and its nature are the same for different samples of seeds of the same species and that the change in solution resistance would be solely dependent upon the change of permeability in the seed. It is also possible that salts clinging to the seeds would affect the results although they had previously been washed with distilled water in the hope of ridding them of all salts.

The direct measurement of tissues or of any tissue as a measure of permeability has been called in question by STILES (**25**, pp. 180–182).

The work with conductance measurements in solution of $KMnO_4$ rather than distilled water offered more promising results. Though there were a

few discrepancies, resistance for the most part rose with germination percentages. Curves plotted from any of the individual tables indicate as great a degree of consistency as most workers seem to obtain from other methods. Seeds of high germination percentages repeatedly exhibit a proportionately high resistance in solution.

By far the simplest method evolved in these experiments on viability was the comparison of the time element in the reduction of $KMnO_4$ with viability. It is obvious that seeds of low viability exhibit the property of reducing the potassium permanganate in less time than is required by seeds of higher viability. It does not matter whether the seeds of zero germination have been killed by heat, frost, disease, or have died of old age. They are nearly always the first to reduce the permanganate. It is possible to employ this method for determining seeds of high, low, and medium viability, but like other methods mentioned above, it needs considerable refinement before it will show differences in seeds varying 2 or 3 per cent. in germination.

Summary

1. A review of the literature on the various methods for determining viability of seeds is presented.

2. A new and very simple method of determining viability of seeds is recommended. It is useful for seed testing and, as in other methods mentioned, in classifying seeds of high, low, and medium viability only. It is not acceptable when difference in germination of two or three per cent. is desired, and we have still to hunt for a means of obtaining this degree of accuracy. The method suggested consists in determining the time-rate of reduction in $KMnO_4$ solution in which the seeds are soaking.

3. Proof is furnished that the substance which reduces the $KMnO_4$ is not an enzyme and that it may belong to the group of substances known as amino acids, peptides, and amides.

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BRIEF PAPERS

PLANT PHYSIOLOGY IN RUSSIA

(WITH TWO FIGURES)

In visiting the principal agricultural experiment stations and universities on a trip through European Russia during the summer of 1927, the author was impressed by the especially advantageous position of the plant physiologists there, and with the fine quality and great extent of the research in progress. American physiologists have known of the high regard among Russian people for TIMIRIAZEFF and PALLADIN. Through the accomplishments of these men and of contemporaries, plant physiology enjoys a standing which may well be emulated by us. There is no tendency to place this subject in a subordinate position, as is so frequently done in America, where physiological research is often under the direction of a pathologist or crop specialist with strong political or economic connections. The Russian physiologist is concerned with solving fundamental principles in physiology and is by no means held to the solution of problems of immediate commercial application or routine work as many plant physiologists are in the United States.

Work on respiration and upon enzyme action is being prosecuted by Dr. S. KOSTYTSCHEFF with a staff of research workers in spacious and well-equipped laboratories of the Academy of Science in Leningrad. The annual budget for supplies and equipment might be envied by most of our experiment station physiologists. Work on photosynthesis seemed everywhere to be in progress, and well equipped. Dr. N. A. MAXIMOW and Mme. KRASSNOSELSKIA-MAXIMOW had extensive equipment at the Detsko Selo station. (See fig. 1.) They had just completed an important phase of their work on the photosynthetic rate. At the Botanic Garden in Leningrad Dr. LUBIMENKO with several assistants was conducting work on this same problem and upon the quantitative relationship between chlorophyll content and photosynthesis. He has devised an excellent spectrocolorimeter method for the quantitative determination of chlorophyll. Both of these laboratories were equipped for the control of light, temperature, and humidity. Students from these laboratories were busy also at the biological station at Alexandrovsk on the Arctic Sea.

The physiological laboratories of the Institute of Applied Botany at Detsko Selo under Dr. MAXIMOW are well equipped also for work on root physiology and development. Mme. HELEN KRASSOVSKY is principally concerned with this phase. Work on drought resistance is of major impor-

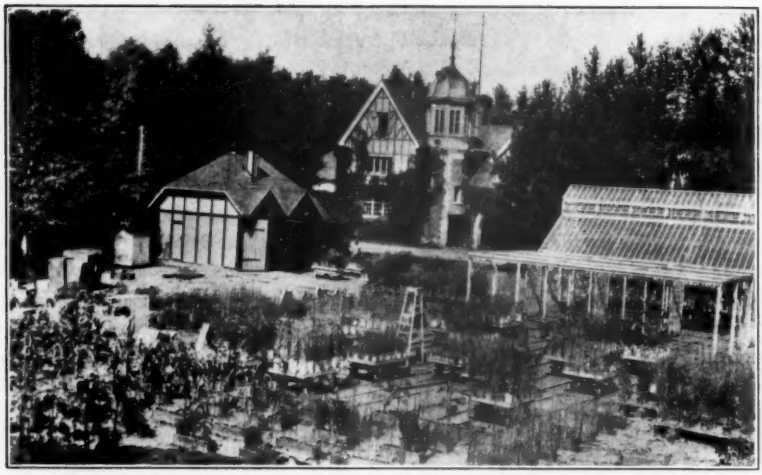

FIG. 1. Dr. MAXIMOW'S experimental greenhouses, plots, and photoperiodism work, and drouth resistance experiments. Photograph by HARVEY.

tance in Russia. Dr. MAXIMOW and Mr. IVAN TUMANOV were giving much time to it. Work on the low temperature resistance of plants was being greatly increased through the installation of three refrigerating machines of the Audfren Singrun type, and by the construction of low temperature greenhouses. Miss LEBEDINZEV was working especially on the dilatometer method of determining free and bound water in plants. Work on photoperiodism at Detsko Selo had close correlation with the cytological work of Dr. LEVITSKY. All of the physiological work enjoys the best of relations with the plant breeding department under Dr. PISSAREFF, and with the soil nutrients work of Drs. GLINKA and KASANOFF. (See fig. 2.)

Field stations for supporting the work on frost resistance, drought resistance, and photoperiodism have been established all over Russia from west to east, and from the Black Sea to Kola. These are established at short intervals of latitude and give a great range of frostless period, length of day, and of other climatic conditions. An unparalleled series of meteorological observations are taken at each station. It is to be regretted that meteorological stations in the United States are not as well correlated with such work in agriculture. The meteorological stations in the Hibini Mountains determine all the weather conditions at five altitudes up to 3,000 feet. The instruments are of the most recent type and readings are checked by two or three kinds of instruments, thus giving a comparison between types of record for the same ecological factor. At Hibini ten meteorologists were on duty.

FIG. 2. Dr. KASANOFF and the mineral nutrient experiments at the Agricultural College at Detsko Selo. Photograph by HARVEY.

The work of the Institute of Applied Botany under the active leadership of Dr. VAVILOV has concerned itself greatly with the physiological problems related to plant geography and plant introduction.

One gets the impression of a thoroughly conscientious and helpful governmental support for fundamental research throughout Russia. The lack of organization of the work in plant physiology and geography in our own Bureau of Plant Industry is certainly in strong contrast with the fine conditions given by the Bolsheviki for these phases of research. Very few of the scientists in Russia are Communists, yet their work is given excellent support. The scientist is left alone by the politicians. If our government is ever to give adequate support to the fundamental and necessary work in plant physiology, the research organizations supported by the government must likewise be entirely free from political and unsympathetic management.—R. B. HARVEY, *Cambridge University, England.*

SOIL MOISTURE AT PERMANENT WILTING OF PLANTS

The residual moisture in the soil when plants attain the condition known as permanent wilting has been the subject of much work and discussion among those interested in plants and soils. BRIGGS and SHANTZ held that when a given soil reached a certain moisture content, plants growing thereon wilted, and could not be revived until additional water

was added to the soil. CALDWELL, and SHIVE and LIVINGSTON, disagreeing with this conception, held that permanent wilting depended upon atmospheric evaporating conditions. BRIGGS and SHANTZ further held that the wilting coefficient of a given soil could be calculated from the moisture equivalent without the necessity of a direct determination by actually wilting the plants. As a result of some 1300 trials with 20 soils they reached the conclusion that the wilting coefficient could be obtained by dividing the moisture equivalent by the factor 1.84.

In extensive experiments extending over a period of years, the writers have observed a remarkable constancy of the residual moisture content for a given soil when permanent wilting is attained under widely varying evaporating conditions. While most of the work was done with sunflowers, a number of trials with other plants seemed to substantiate the contention of BRIGGS and SHANTZ that on a given soil all plants reduce the moisture content of the soil to about the same extent when permanent wilting is attained. In an earlier report[1] the writers showed that with some soils there was a remarkable agreement between the observed and the calculated wilting coefficient. However, later results with many kinds of soils do not uphold the correctness of the 1.84 ratio, and we do not believe that a common factor for all soils may be used to calculate the amount of water which remains in the soil at permanent wilting.

The plants were grown in two sizes of containers under the varied seasonal atmospheric conditions which prevail at Davis, California. Maximum temperatures varied from about 45° F. during the winter months in an unheated greenhouse to 115° F. out-of-doors during the summer. Evaporation during these periods from a white spherical atmometer varied from 4 or 5 cc. to 80 cc. for 24 hours. A full description of the experiments will be published later. Some of the results obtained in over 2000 trials with 29 soils are given in the acompanying table.

It will be seen at once that the ratios range from 1.73 to 3.82. The results show clearly that there is no relation between the ratios obtained and the type of soil used. No logical grouping is possible because some of the highest as well as some of the lowest ratios were found within a given classification of soils. For example, high and low ratios were found with sands, with loams, and with clays. It would thus seem from the data obtained with the soils and plants used in these experiments that the residual moisture at permanent wilting cannot be obtained in every case from the moisture equivalent by use of the factor 1.84.

[1] VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil Moisture conditions in relation to plant growth. Plant Physiol. 2: 72–81. 1927.

TABLE I

RESIDUAL MOISTURE AT PERMANENT WILTING AS DETERMINED WITH SUNFLOWER PLANTS OCTOBER, 1927, TO JUNE, 1928, AT DAVIS, CALIFORNIA

SOIL	NUMBER OF TRIALS	*MOISTURE EQUIVALENT	* PERCENTAGE OF MOISTURE IN SOIL AT PERMANENT WILTING	RATIO OF MOISTURE EQUIVALENT TO MOISTURE AT PERMANENT WILTING
N	18	3.29 ± 0.022	1.41 ± 0.031	2.33 ± 0.054
U	16	6.05 ± 0.036	3.50 ± 0.030	1.73 ± 0.018
HS–2	35	8.32 ± 0.033	3.67 ± 0.020	2.27 ± 0.015
HS–1	35	8.84 ± 0.041	3.99 ± 0.030	2.295 ± 0.020
FS	226	10.50 ± 0.026	3.08 ± 0.007	3.41 ± 0.011
TL	78	13.71 ± 0.021	4.51 ± 0.020	3.04 ± 0.014
J	17	17.07 ± 0.017	6.15 ± 0.070	2.775 ± 0.013
Y	40	17.16 ± 0.033	8.82 ± 0.027	1.95 ± 0.007
TC	24	17.30 ± 0.044	7.89 ± 0.040	2.19 ± 0.012
S	39	21.35 ± 0.043	10.20 ± 0.029	2.09 ± 0.007
OL	27	23.36 ± 0.052	6.12 ± 0.033	3.82 ± 0.022
OC	29	24.51 ± 0.050	11.55 ± 0.059	2.12 ± 0.012
MG	151	25.63 ± 0.039	10.47 ± 0.025	2.45 ± 0.007
V	24	37.90 ± 0.067	19.03 ± 0.074	1.99 ± 0.008

* Calculated on a dry weight basis.

While the residual moisture content of some soils at permanent wilting shows close agreement with the 1.84 ratio, as the writers have previously shown, the general use of this ratio for all soils is open to serious criticism. From the foregoing data, it is evident that the amount of water available for plant growth cannot be determined from the moisture equivalent alone. In the opinion of the writers, the moisture equivalent is the best single-value determination for interpreting the moisture properties of soils, but it is not an exact measure of how much of that water is available to growing plants. For accurate work it is evident that the amount of readily available moisture can be obtained only when the amount of residual moisture at permanent wilting is known, because it seems that plants are able to reduce the moisture content of different soils to different degrees of dryness before this stage of wilting is reached.—F. J. VEIHMEYER AND A. H. HENDRICKSON, *University of California.*

NOTES

Officers for 1928–1929.—The Secretary of the American Society of Plant Physiologists has announced the results of the recent annual election as follows: For President, Dr. E. J. KRAUS, University of Chicago; Vice-President, Dr. SCOTT V. EATON, University of Chicago; Secretary-Treasurer, Dr. H. R. KRAYBILL, Purdue University. The Society is in position now to go forward with great confidence in the future. The budget for the publication of PLANT PHYSIOLOGY has been balanced during the last two years, and the normal growth should continue at a rate sufficient to provide for enlargement of the publication program from time to time. All members will want to cooperate fully with the officers in planning for the New York meeting, and a period of rapid advancement is anticipated for the coming year.

Sixth National Colloid Symposium.—The sixth National Colloid Symposium of the American Chemical Society was held in Canada at the University of Toronto on June 14–16, 1928, with Sir WILLIAM HARDY, of Cambridge, England, as the guest of honor. About 150 members registered, and there was a large number of visitors from Toronto at the meetings. Twenty-six papers were scheduled for the three days.

Sir WILLIAM HARDY, in his opening address, spoke of the relation of colloidal behavior to biological problems, asserting his belief that the next great advance in science would be in the field of biology.

There were few papers of direct interest to plant physiologists, both soil and plant colloids being rather neglected. The program, however, was varied, and brought into prominence the wide range of colloidal applications. Electrical relations were dealt with in several papers, but Dr. HARKINS was unavoidably absent, and unable to introduce the topic. Adsorption, at the hands of Professors MCBAIN and BANCROFT, was given a considerable amount of discussion, Dr. BANCROFT amusing his hearers with his well-known aversion to "activity." Rubber, medicine, bacteria, explosives, and photography were among the subjects which received attention. Possibly of greatest interest to the plant physiologist was the paper by Professors WASTENYS and BORSOOK, of the University of Toronto, on the enzymatic synthesis of protein, with its suggestion of a method by which different proteins are synthesized *in vivo*. The utilization of moving pictures in ultramicroscopic fields was a noticeable feature of the program.

The city of Toronto provided many points of interest. At the University, the Connaught Laboratories and the science buildings were open to inspection. Among the experiments shown was a successful run for the

liquefaction of hydrogen and helium. For entertainment, golf was arranged on a variety of courses, and the swimming pool in Hart House was an attraction. The annual banquet was held on Friday in the Great Hall, and few will ever forget the delightful costumes and the excellent music of the sextette of "Canadian Singers" after the dinner, and later in Hart House theater.

International Nitrogen Conference.—The second International Nitrogen Conference was held on board the Steamship *Luetzow*, on the Adriatic Sea, May 1–5, 1928. It was sponsored by the nitrogen fertilizer interests of France, Italy, England, Norway, and Germany. Some well-known names appear among the speakers and attendants at the meeting. Addresses were made by Sir FREDERICK KEEBLE, Dr. ERWIN BAUR, Prof. L. BRETIGNIÈRE, Dr. A. DEMOLON, Dr. J. BUEB, Prof. FIRMAN E. BEAR and others. Participating in the discussion were such noted figures as Sir DANIEL HALL, and Prof. HENRY ARMSTRONG, of England. Fifteen countries were represented. With the discovery of processes for making synthetic fixed nitrogen cheaply, a vast nitrogen fertilizer industry has sprung up which is destined to play an important part in the permanent fertility of agricultural lands. It is also probably destined to figure prominently in the commercial field, with a tendency toward vast industrial monopolies, with international understandings and agreements as to distribution and prices of nitrogen fertilizers. The commercial significance of the meeting probably outweighs its scientific significance.

Seeds For Investigational Purposes.—Since much work needs to be done on the physiology of trees, sources of tree seeds are important to the investigator. The seeds of many native trees and woody shrubs exhibit delayed germination. Otto Katzenstein and Co., 6 Cone St., Atlanta, Georgia, handle seeds of many of our native trees and shrubs, and the seeds of such cultivated fruits as apples, apricots, cherries, lemons and oranges, pears, persimmons, plums, and quinces. They also carry roots and seeds of medicinal plants. Thirty years of experience in this field makes possible excellent service to those who need seeds for experimental purposes.

Books of Historical Value.—Occasionally one finds it desirable to obtain books which have historical value. Not long since it was possible for the writer to secure a good copy of JETHRO TULL'S Horse-Hoeing Husbandry, 1733, from THOMAS THORP, Strathfieldsaye, Guildown Road, Guilford, England. This work had been sought for several years without success. Others who desire old books might find it worthwhile to submit want lists to Mr. THORP.

Summer Meeting at Purdue University.—Plans for the summer meeting at Purdue University have been formulated by the Secretary-Treasurer in cooperation with the Purdue Section. On September 4, a number of papers will be presented by members of the Purdue Agricultural Experiment Station, dealing with the work of interest to plant physiologists which is now in progress at the station. The following day will be devoted to field excursions and demonstrations. The dinners and luncheons will be made periods of delightful social intercourse, and visitors are assured a most valuable experience. It is hoped that many of the members will find it possible to participate in this meeting.

The Fifth Annual Meeting.—Plans for the fifth annual meeting of the American Society of Plant Physiologists at New York in December, 1928, are rapidly maturing. The program committee is hard at work on the various features of the meeting. The members of the committee should be given hearty support by everyone, and this fifth annual meeting should be made the largest and best meeting in our history. Since active and intelligent discussion of the papers presented adds pleasure and zest to these occasions, those in attendance can help to make the meetings most valuable by engaging in such discussions. The programs should not be so crowded that discussion is precluded. It should also be remembered by those who offer papers to the program committee, that attendance and personal presentation of the papers listed is a courtesy to the program committee and to the Society which should not be omitted. Visitors are sometimes keenly disappointed when papers have to be read "by title only." If attendance is doubtful, it is better not to list papers on the program.

The Stephen Hales Prize.—The committee which was appointed to devise a method of handling the STEPHEN HALES Prize has practically completed its work, subject to the approval of the executive committee, and acceptance by the Society. The committee which is to select the first recipient of the award consists of the following members: Dr. J. B. OVERTON, University of Wisconsin, chairman; Dr. A. L. BAKKE, Iowa State Agricultural College; and Dr. C. R. BALL, of the U. S. Department of Agriculture, Washington, D. C.

Life Membership Committee.—The committee chosen to select the third CHARLES REID BARNES life member has been appointed by the president. The personnel of the committee is as follows: Dr. W. M. ATWOOD, Oregon Agricultural College, Corvallis, Oregon, chairman; Dr. P. D. STRAUSBAUGH, West Virginia University; Dr. E. B. MAINS, Purdue University; Dr. A. E. MURNEEK, University of Missouri; and Dr. H. W.

BROWNING, Rhode Island State College. This committee has one of the most pleasant duties to perform. The report of the committee will probably be made at the annual dinner at the New York meeting.

Membership Committee.—The membership committee for the year 1928–1929 has recently been appointed, and consists of the following members: Chairman, Dr. E. M. HARVEY, Oregon Agricultural College; Dr. A. L. BAKKE, Iowa State College; and Dr. EARL S. JOHNSTON, University of Maryland. Rapid growth can be made if all members assist the committee in its important work. If you know of someone who should be invited to membership, send the name to some member of the committee, or to the Secretary-Treasurer, Dr. H. R. KRAYBILL, Purdue University, Lafayette, Indiana.

Starch Chemistry.—A symposium on the chemistry of starch, and a bibliography of nearly 10,000 titles on this subject has been brought together in book form by ROBERT P. WALTON in collaboration with a group of authors of international reputation. There are nineteen papers in the symposium, some of which deal with the molecular constitution of starch. The first four sections consider thermal depolymerization, methylation, enzyme hydrolysis, and bacterial degradation, with reference to these problems of molecular constitution. Other papers consider the colloidal properties, X-ray spectrography, nature of amylases, rôle of starch in bread making, conversion in the fermentation industries, manufacture of corn and potato starch, production of dextrins and gums, and other adhesives, and the use of starch in the paper and textile industries. The final section considers the historical development of starch chemistry and manufacture. While the price of the book is $10.00 the bibliography is an expensive item in the cost of publication. It is published by the Chemical Catalog Co., New York.

Principles of Plant Physiology.—A new text-book of plant physiology has appeared from the press of the Macmillan Co. The author, Dr. ORAN L. RABER, best known for his interest in permeability, is at present a member of the staff of Biological Abstracts.

The book is intended for elementary classes, but somewhat overshoots the mark so far as the chemistry is concerned. There are thirty-one chapters, some of which are well done, while others are not satisfactory. The order of arrangement is not so very logical. For instance, after the introductory chapters on the sciences and the cell, come chapters on photosynthesis and nitrogen metabolism, and the chemistry of carbohydrates, fats,

waxes, lipides, proteins, miscellaneous products and enzymes, preceding the more readily grasped processes of water absorption, diffusion of gases, osmotic action, transpiration, etc. Aerobic respiration precedes anaerobic in the treatment, although in the actual process it is pretty certain that it starts anaerobically, and becomes aerobic in such organisms as possess the necessary enzymes. The last two chapters have little to do with plant physiology, and the work would not suffer by their omission. The main criticism to be made is the large number of errors. Many of them are small, but it is disappointing to find errors of statement, or errors of judgment, sprinkled freely through the work. Beginning students do not have the necessary background to detect these mistakes, so that the instructor who uses the text will have to watch for the errors involving emphasis, points of view, and essential facts. The need of a good text in this field is very urgent, and RABER is to be complimented for having made the attempt to provide a text. The price of the text is $3.00, and the book can be ordered from the publishers, Macmillan Co., New York.